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INFLUENCES OF TEMPERATURE AND MOISTURE ON HATCHING OF EGGS OF THE PALE WESTERN CUTWORM, *AGROTIS ORTHOGONIA* MORR. (LEPIDOPTERA: NOCTUIDAE)[1]

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ABSTRACT

Laboratory studies showed that hatching of fully developed eggs of the pale western cutworm varied directly with temperature and relative humidity, that prolonged exposure to temperatures from −5° to −15° C. did not affect ultimate hatch, and that desiccation, particularly in the range 20° to 30° C., caused considerable embryo mortality. Studies outdoors showed that 45 to 60 days were required in the fall to complete embryonic development and that most of the hatching occurred in the early spring. Findings in the laboratory, corroborated by studies outdoors, showed that eggs are admirably adapted to develop, withstand climatic factors, and hatch at a time when their survival is ensured.

INTRODUCTION

In Western Canada, moths of the pale western cutworm, *Agrotis orthogonia* Morr., are in flight between August 15 and September 15. Most of the eggs are laid during the middle two weeks of this period. Development of the embryo occurs during the fall and the eggs hatch in late March or early April.

The studies reported herein were made to assess the various climatic hazards that affect hatching and survival of eggs under conditions that prevail in the Canadian prairies.

GENERAL METHODS

Eggs were obtained from females collected on flowers in the field and, during the winter, from females that emerged in the laboratory. Females and males were placed in 1-pint jars containing sifted soil and fed on a 10 per cent honey-water solution. Eggs were obtained daily by sifting the soil through a 32-mesh sieve. After separation from the soil the eggs were cleaned of adhering soil particles and other debris. For studies on embryonic development in the outdoors, eggs were placed in the soil on the day that they were laid. Fully incubated eggs were obtained by incubating them at room temperature or at 30° C.; after incubation they were stored at 0° and 5° C. until needed.

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Groups of fully developed eggs were placed at 0° C. on November 1, 1954, and brought out to hatch at monthly intervals as checks to be compared with the hatching of eggs in the plots. From December 1 to March 1 the percentage of these eggs hatching varied from 92 to 99.

In laboratory studies, each test involved four replicates of 10 or 25 eggs, and the eggs of each replicate were placed in glass vials 3/4 inch in diameter by 1 inch in height. The hatch was recorded 4, 8, and 12 hours after, or at the beginning of, the treatment and then at daily intervals until completed. Constant relative humidity was maintained in 1-pint sealers containing calcium chloride for the lowest humidity, distilled water for a saturated atmosphere, and various concentrations of sulphuric acid for the intermediate humidities. Each sealer was covered with half of a petri dish that was sealed to the top edge with petroleum jelly. The tubes containing the four replicates were supported above the calcium chloride, water, or sulphuric acid on a platform made from the bottom of a 15– × 60–mm. petri dish cemented to a 50-ml. beaker.

Constant temperatures were maintained in controlled temperature rooms in which variation was within 1 degree.

In a plot outdoors, four replicates of 10 to 25 eggs were used in each test, and the eggs of each replicate were placed in 1-inch cubic plastic boxes. A hole 1/2 inch in diameter was drilled in the lid and in the bottom of the box and fitted with a square piece of 100-mesh bronze screening. The box was filled with sifted soil to within 1/4 inch of the top. The eggs were distributed evenly on the soil surface and covered with soil to the top edge. The lid was then pressed on tightly. The plastic boxes were placed in soil in the plot outdoors, the eggs being placed at the desired depth. For studies on embryonic development the boxes were removed after various periods and development was determined by microscopic examination and by noting the time required to complete development at 30° C. For studies on hatching, fully developed eggs were placed at a depth of 1/2 inch in the plot at monthly intervals from November 1 to February 1 and brought into the laboratory for hatching after various periods until April 1. The numbers that had hatched outdoors were recorded for each replicate and the remaining eggs were hatched in petri dishes containing disks of blotting paper that were moistened daily.

Notes were made on the weather, the amount of snow cover, and other pertinent information on the plot area for the period during which the eggs were in the soil. Temperatures in the plot were measured by thermocouples and recorded on a Brown recorder.

LABORATORY STUDIES

Replicated series of 25 eggs were placed at various temperatures and humidities to hatch. Figure 1 shows that at 25° C. the rate of hatching of fully developed eggs varied directly with the amount of moisture in the atmosphere. The times required for 50 per cent hatch, determined from probit regression lines, were 3.2, 2.5, 1.7, and 1.0 days at 25, 50, 75, and 100 per cent R. H., respectively. There was a marked difference in the numbers hatching between eggs kept at 0 and at 25 per cent R. H. or higher, indicating that moisture ensures a relatively high hatch. At 0

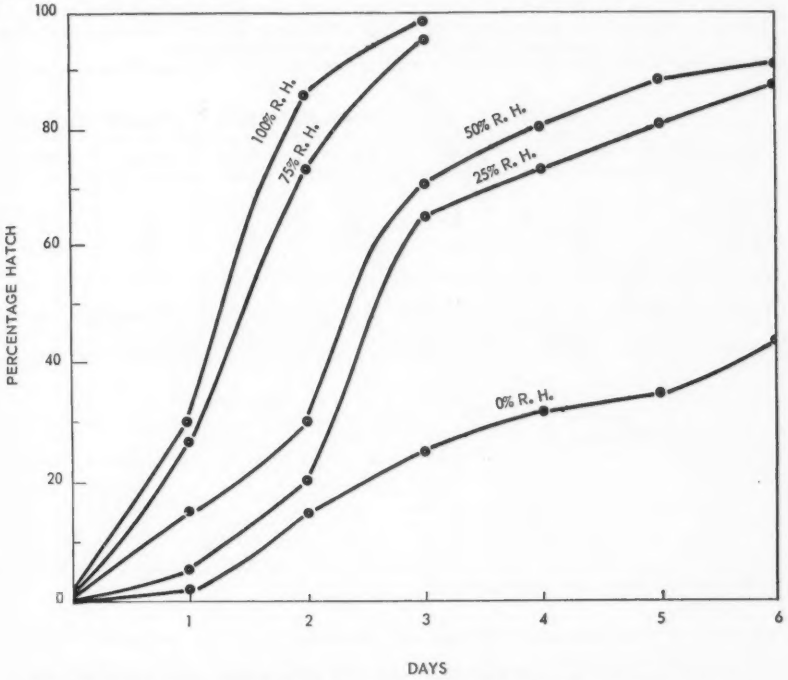

FIGURE 1. Percentages of fully incubated eggs of the pale western cutworm hatching at various relative humidities at 25° C.

per cent R. H. only 41 per cent of the eggs hatched after 6 days, but when the unhatched eggs were placed on the surface of moistened blotting paper in petri dishes all hatched within 24 hours.

Another test showed no difference in the rate or numbers hatching between eggs that were moistened and eggs that were held in a saturated atmosphere.

Figure 2 shows that the rate of hatch of fully incubated eggs varied directly with temperature as well as with relative humidity. The times required for 50 per cent hatch at 30°, 25°, and 20° C. when moisture was present did not differ greatly. At 15°, 10°, and 5° C. the difference was most marked between the low and medium humidity. At desiccation fewer than 50 per cent hatched at temperatures below 15° C.

As shown in Figures 1 and 2, lack of atmospheric moisture at all temperatures delayed and reduced hatching. Dissection showed that many of the embryos were dead, especially at 25° and 30° C., where the total hatch was less than 50 per cent.

To determine the effect of desiccation replicated series of 10 eggs were placed at 0 per cent R. H. and 30°, 25°, and 20° C. and removed as follows: from 30° C. after 4, 8, and 12 days; from 25° C. after 4, 8, 10, and 11 days; and from 20° C. after 4, 8, 12, 14, and 16 days. While the eggs were at 0 per cent R. H., hatching was recorded daily and the larvae were removed.

TABLE 1.—HATCH AND MORTALITIES OF FULLY DEVELOPED EGGS OF THE PALE WESTERN CUTWORM AT 0% R.H. AT VARIOUS TEMPERATURES

Temperature	Total percentage hatch	Hatching period	Percentage mortality of unhatched eggs after			
			4 days	8 days	11 days	14 days
30° C.	28	3	21	97	—	—
25° C.	45	5	10	46	83	—
20° C.	56	7	—	24	30	81

TABLE 2.—PERCENTAGES OF EGGS OF THE PALE WESTERN CUTWORM HATCHING AFTER VARIOUS PERIODS AT THREE LEVELS OF RELATIVE HUMIDITY AT 0° C.

Relative humidity, %	Days at 0° C.		
	90	118	149
0	82	56	20
50	91	83	65
100	97	95	74
Difference necessary for significance at 5% level	10	15	11

On removal after the various intervals the unhatched eggs were placed in contact with moistened blotting paper in petri dishes and the mortality from desiccation determined from the numbers that failed to hatch.

Table 1 shows that the maximum period of tolerance of fully developed eggs without appreciable mortality to 0 per cent R. H. was less than 4, 8, and 12 days at 30°, 25°, and 20° C., respectively. As the temperature was lowered the deteriorating effect of desiccation lessened, presumably because the saturation deficiency was lower. In another test, all the embryos at 10° C. and half of those at 5° C. were dead after 48 days at 0 per cent R. H.

Desiccation at 0° C. was also harmful but the effect was not noted until after 90 days and it increased markedly from that time on (Table 2). The difference between the numbers hatching at 0 per cent R. H. and 100 per cent R. H. was not statistically significant until the 90th day. The difference between the numbers hatching at 50 per cent and 100 per cent R. H. was not significant until the 118th day.

Fully developed eggs were placed at several temperatures below 0° C. for various intervals. Successive trials showed that these eggs could not withstand −20° C. longer than 36 hours. No harmful effects were found in eggs stored at −15° C. for 120 days.

OUTDOOR STUDIES

Development and hatching studies were conducted outdoors in the laboratory plot during two fall and winter seasons, 1954-1955 and 1955-1956.

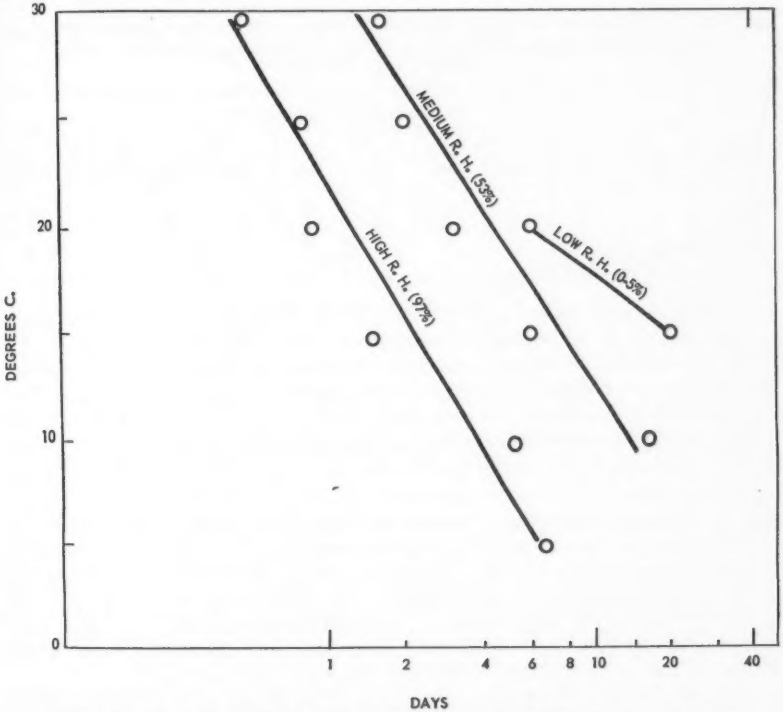

FIGURE 2. Times for 50 per cent hatch of fully incubated eggs of the pale western cutworm at three levels of relative humidity at various temperatures.

The selection of these years was fortunate, as they represented extremes of winter climate in southern Alberta. The 1954-1955 period was marked by a very mild and dry interval from September to January followed by light snows in January and February with frequent periods of above-freezing temperatures from January to the end of March. During the 1955-1956 period September was very warm and was followed by warm, windy weather in October. About November 10 the first heavy snow of the year occurred. This was not dissipated until spring and temperatures were uniformly cold until late in March. Temperatures in the soil from October 1 to April 10 are shown graphically for the two years in Figure 3 along with the intervals when maximum soil temperatures were below freezing.

TABLE 3.—MEAN PERCENTAGES OF DEVELOPMENT OF EGGS OF THE PALE WESTERN CUTWORM PLACED OUTDOORS IN 1955 FOR VARIOUS PERIODS, LETHBRIDGE, ALTA.

Date eggs placed in soil	Percentage development after				
	10 days	20 days	30 days	40 days	50 days
Aug. 26	50	55	80	100	—
Sept. 6	45	55	75	90	95
Sept. 26	25	55	75	90	95

TABLE 4.—PERCENTAGES OF FULLY DEVELOPED EGGS OF THE PALE WESTERN CUTWORM HATCHED WHEN HELD OUTDOORS FOR MONTHLY PERIODS, LETHBRIDGE, ALTA., 1954–55 AND 1955–56

Date placed in soil	Percentage hatch when removed on									
	Dec. 1		Jan. 1		Feb. 1		Mar. 1		Apr. 1	
	1954	1955	1955	1956	1955	1956	1955	1956	1955	1956
Nov. 1	0	3	0	4	2	4	16	8	63	34
Dec. 1	—	—	32	0	36	0	59	0	59	4
Jan. 1	—	—	—	—	5	0	2	0	36	0
Feb. 1	—	—	—	—	—	—	17	0	26	0

Table 3 shows that in 1955 about 40 to 60 days were required from oviposition until embyronic development was completed outdoors. In 1954, eggs placed in the soil outdoors required 55 days for development. During that year slight differences in development were found in eggs that were placed at depths of 1/4 inch and 1 inch. At a depth of 1/4 inch the time to complete development was about four-fifths of that at the 1-inch level. This reflects the difference in temperature at these levels. Table 4 shows that most of the fully developed eggs that hatched after being placed outdoors did so in late March and early April, and hatching was completed in both years by April 16. The hatching during each winter was associated with periods of mild weather. Hatching during December of 1954 was the result of unseasonably warm weather. In 1955 some hatching occurred in early November until onset of subzero temperatures and no further hatching in the plot was found until after March 15, when the snow cover was gone and temperatures in the soil had risen above 0° C.

During a 4-year period commencing in 1947 the first spring hatching outdoors occurred between March 2 and March 29.

DISCUSSION

Lindsay (4) has shown that at constant temperatures the period of embryonic development of the pale western cutworm varies from 11 days at 30° C. to over 100 days at 10° C.; in the range 15°–20° C. from 20 to 35 days are required to complete development. Eggs were fully developed outdoors after a period of about 40-60 days (Table 3). In years of rain and prolonged cool weather the developmental period would be lengthened. In most years on the Canadian Prairies there is time to complete most of the embryonic development before winter. Lin, Hodson, and Richards (3) have shown that short periods of developmental temperatures are utilized in an accumulative manner. Hence, if development has been retarded or interrupted by cool weather, it can be resumed intermittently and the eggs can be ready for hatching by spring.

Eggs can withstand up to 120 days at −15° C. without seriously affecting the hatching. Figure 3 shows that, because of the insulating effect of snow and soil, soil temperatures at 1/2 inch rarely go below −10° C. for sustained periods. Therefore, it is concluded that freezing temperatures are not a serious hazard to the survival and hatching of the eggs.

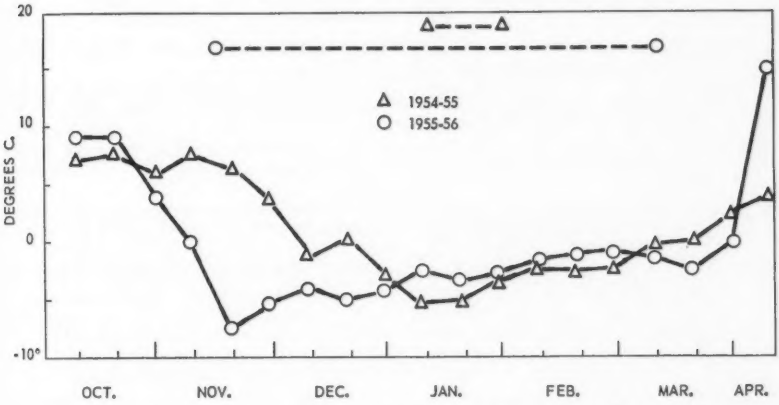

FIGURE 3. Mean soil temperatures at a depth of 1/2 inch from October to April in two years, Lethbridge, Alta.; *broken lines*, maximum soil temperatures below freezing.

Hatching in the fall has been reported by Cook (1) and by Parker, Strand, and Seamans (5). Reports of larvae surviving the winter are meagre, and Cook (1) states that hatching in the fall is abnormal. As shown in Table 4, some hatching occurs in the fall and also during the winter when the weather is mild, but most of the eggs do not hatch until late in March or April. The rate of hatching increases as the temperature is raised and proceeds more slowly at each temperature as the relative humidity is decreased. Low temperatures, accompanied at times by low moisture, prevail from November to March in the prairie regions of Western Canada. Thus, opportunity for complete hatching does not usually occur until spring.

Desiccation in the range 20°–30° C. can delay hatching and also cause mortality of the embryos (Table 1), but there is little likelihood of such conditions occurring outdoors for extensive periods. However, when eggs are kept for prolonged periods at temperatures as low as 0° C. and low relative humidities the ultimate hatch is reduced (Table I). Hodson and Weinman (2), in investigations with eggs of the forest tent caterpillar, found that low relative humidities prevented hatching and caused considerable mortality of the embryos. They attributed this to two possibilities first, alteration of the chorion to the point where the larvae are unable to break it and, second, mortality of the larvae from too great a loss of water.

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THE EFFECT OF IRRIGATION ON YIELD AND MALTING QUALITY OF BARLEY IN SOUTHERN ALBERTA[1]

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ABSTRACT

Comparisons were made of the yield and malting quality of Montcalm barley grown on dryland and on irrigated plots in two different rotations at the Experimental Sub-station, Taber, Alberta. A supplementary study of the effect of fertilizer was introduced into the experiment. Irrigation significantly increased the yield of grain and improved malting quality, mainly by increasing extract. An increase in yield was also shown on fertilizer treated plots under irrigation, but the amount of increase depended on environmental factors associated with the crop rotation system. The effect of fertilizer on malting quality was insignificant.

INTRODUCTION

It is quite possible that malting barley could be grown profitably as a supplementary crop in some of the irrigation districts in southern Alberta. It is also possible that suitable varieties may have to be developed for this purpose. Since information on the effect of irrigation on malting quality of existing varieties was lacking, this study was undertaken to determine the effects of irrigation on those properties of Montcalm barley having a direct bearing on its malting qualities.

The study included quality tests of barley samples grown with and without irrigation at the Experimental Irrigation Sub-station at Taber, Alberta, during the 5-year period 1949 to 1953.

Taber is located about one-third of the distance between Lethbridge and Medicine Hat and is in the Chinook belt of southern Alberta, within the low-rainfall high-temperature area of the Prairies. As a result, irrigation is usually needed to secure satisfactory yields.

WEATHER CONDITIONS DURING TEST PERIOD

Extremes in weather occurred at Taber during the 1949 to 1953 period as, for example, during the last 3 years when high precipitation, low evaporation, and relatively low temperatures combined to reduce the consumptive use of water by crops. In that period the need for irrigation, especially for short-season crops like barley, was largely and at times entirely eliminated. More nearly normal conditions for the area prevailed during 1949 and 1950 when precipitation was low enough to afford some degree of control over the amount of moisture received by the crops under test.

Precipitation during 1951 was extremely high and resulted in a heavy carry-over of soil moisture into the following year. The total rainfall in 1952 was nearly normal but its favourable distribution and the high soil

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TABLE I.—SUMMARY OF EXPERIMENTAL RESULTS ON THE EFFECT OF IRRIGATION ON YIELD AND MALTING QUALITY OF MONTCALM BARLEY

Means based on 1949, 1950 and 1953 data*

Data recorded	Non-legume Rotation G				Legume Rotation B			
	Non-irrigated		Irrigated		Non-irrigated		Irrigated	
	O	X	O	X	O	X	O	X
Consumptive use of water (inches)	8.5	8.0	15.5	15.8	9.9	9.8	17.2	17.4
Yield per acre (bushels)	23.0	27.9	40.4	46.6	30.2	25.8	57.5	60.5
1000-kernel weight (grams)	32.5	31.6	34.7	34.8	31.5	32.0	35.2	35.2
Barley nitrogen %	2.08	2.02	1.72	1.72	2.21	2.31	1.78	1.78
Malt extract %	76.5	76.3	78.6	78.9	75.8	75.3	79.2	79.3
Malt saccharifying activity	161	154	136	134	168	186	138	132

* The differences between all corresponding means for irrigated and non-irrigated plots in this table were statistically significant at the 5 per cent point.

"O"—Non-fertilized

"X"—80–100 lb./acre (11–48–0) applied at seeding time

moisture eliminated the need for irrigation on the barley plots. The 1953 season was dry and hot during July and August but nearly 10 inches of rain fell in the spring months and only limited irrigation was required to produce maximum yields of barley.

The results obtained for the years 1949, 1950 and 1953 are included in the summary (Table I) of this study. The results from 1952, when no irrigation was needed, are not included, and the 1951 crop was destroyed by a severe hail storm in mid-July.

MATERIALS AND METHODS

Montcalm barley was included in three rotations used in the irrigation studies at Taber. The rotations were:

Rotation B—barley and sweet clover, sweet clover and green manure, sugar beets, corn.
Rotation G—sugar beets, wheat, oats, barley.

The results from the third rotation, C, are not included in this summary, since one range in this rotation was affected by seepage from a shallow drain ditch.

The crop in each rotation consisted of a range of ten plots, each 32 feet square. One-half of each plot was treated at seeding-time with ammonium phosphate fertilizer (11–48–0) at the rate of 80 to 100 lb. per acre. It was planned to use four different irrigation treatments in addition to a check receiving no irrigation, randomized in duplicate series throughout the plots in each range. The treatments were to be dependent on total available soil moisture content. It was not, however, always possible to follow the planned treatments exactly, and in practice irrigation treatment depended upon the amount of rainfall received.

Duplicate yield samples were harvested from each plot unit; allowed to dry for 3 or 4 weeks, and threshed; and the yields per acre determined. Test samples of about 600 grams were taken, and these were malted and analysed by standard procedures in the barley laboratory in Winnipeg. The study concerning malting quality terminated after the 1953 harvest due to the transfer of the irrigation cultural experiments from Taber to Vauxhall, Alberta.

RESULTS AND DISCUSSION

A summary of the results obtained is given in Table I, which shows the amounts of water used, yields of barley per acre and several barley and malt analytical properties obtained in non-legume rotation G and legume rotation B, irrigated and non-irrigated, and with and without fertilizer.

Yield data and the results of malting tests showed marked differences between irrigated and non-irrigated plots. Fewer differences were recorded between the four irrigation treatments; and for the purposes of this summary the results from non-irrigated plots are compared with results from all irrigation treatments. While the irrigated plots covered a rather wide range of moisture, the conditions might be considered generally comparable to field irrigations where enough water is applied at any one time to wet the soil to the depth of the root zone.

Statistical analyses of yield data showed that the yield of barley was significantly increased on the irrigated lots. The fertilizer treatment employed also increased yield to some extent when combined with water application, but the amount of increase depended on environmental factors associated with the crop rotation system.

Malting quality evaluation was facilitated by the fact that Montcalm barley is recognized as being equal to O.A.C. 21, the Canadian standard of malting quality. Detailed investigation of malting behaviour was, therefore, not necessary and comparisons of certain key properties sufficed to determine the effects of irrigation on quality. The specifications for good malting quality in barley are rather loosely defined, but in general include a low barley nitrogen content, a medium sized kernel and a high malt extract value. The results of this investigation show that irrigation invariably lowered the barley nitrogen content and significantly increased the malt extract value. The weight per thousand kernels was increased, but not to the extent that the kernel could no longer be considered medium sized. A fourth factor, the saccharifying activity of malt, was lowered by irrigation treatment. The increases in malt extract and kernel weight, and the decreases in nitrogen content and saccharifying activity due to irrigation, were all statistically significant. The desired level of saccharifying activity of a malt is not clearly specified, but present industrial opinion indicates that, since Montcalm possesses ample saccharifying power, a reduction of the order in these experiments is not detrimental to quality. The net result of irrigation is, therefore, an improvement in the malting quality of Montcalm barley, primarily by increasing malt extract. It is reasonable to assume that other malting barleys, such as O.A.C. 21 and Olli, would be similarly affected. No significant effects on malting quality due to fertilizer were found in this study.

The success of an irrigation experiment depends upon the consistency of the natural precipitation during the course of the test. Due to the abnormalities of 1951 and 1952, the study of the effects of irrigation on yield and quality is not as complete as might be desired. Nevertheless, it is felt that valuable indications of what might be expected from the use of irrigation in barley production have been obtained.

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THE EFFECT OF NITROGEN AND PHOSPHORUS FERTILIZERS ON THE YIELD AND PROTEIN CONTENT OF SPRING WHEAT GROWN ON STUBBLE FIELDS IN SOUTHERN ALBERTA[1]

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ABSTRACT

Varying rates of nitrogen and phosphorus were applied to hard red spring wheat grown on stubble fields at three locations in southern Alberta in 1955 and 1956. Significant yield increases were obtained at the three locations in 1955 as a result of applications of nitrogen. At two of the three locations further increases in yield resulted from the addition of phosphorus. In 1956 significant yield increases were obtained at two of the three locations following nitrogen applications, and at only one of these locations did the addition of phosphorus result in further significant increases in yield. At two of the three locations in both 1955 and 1956 high rates of nitrogen fertilizer caused significant increases in protein content over the unfertilized check. Phosphorus additions significantly reduced the effect of nitrogen on the protein content at one location in one year, but had no consistent effect in the other experiments. The experimental results indicate that, when moisture is adequate, more than 40 lb. of nitrogen per acre, and at least 80 lb. per acre in some cases, must be applied before increases in protein content of spring wheat can be expected.

INTRODUCTION

The mean protein content of the hard red spring wheat crop of Western Canada has been lower in recent years than the long-term average (3). As a result, a great deal of interest has been shown concerning the possibility of raising the protein content of spring wheat by fertilization.

The influence of fertilizers on the growth and composition of wheat has been studied extensively with varying results. In Western Canada it has been reported (7, 14) that fertilization has not affected the protein content of spring wheat although yields have been increased in some cases. Reports from the United States indicate that the application of nitrogen at the time of seeding, or early in the season, has resulted in increased yields without affecting the protein content of the grain, whereas nitrogen applied at or near heading time has resulted in higher protein content without affecting yield (4, 6, 10). However, other investigators (2, 8, 15, 16) have noted increases in yield along with higher protein content resulting from applications of nitrogen fertilizer at the time of seeding spring wheat. Similar increases in protein content have also been shown for other cereals (5, 9, 11, 12, 13).

This paper reports data on the yield and protein content of hard red spring wheat grown on stubble fields in southern Alberta during 1955 and 1956 when nitrogen and phosphorus were applied at varying rates.

MATERIALS AND METHODS

In 1955 the experiments were located at Foremost in the Brown Soil Zone of Alberta (19) and at Nobleford and Claresholm in the Dark Brown Soil Zone (18). In the 1956 experiments Pincher Creek, in the Shallow

[1] Joint Contribution from the Field Husbandry Division and the Illustration Stations Division, Experimental Farms Service.

TABLE 1.—PRECIPITATION DATA AT THE EXPERIMENTAL LOCATIONS

Location	April	May	June	July	Yearly total	April	May	June	July	Yearly total
	in.	in.	in.	in.	in.	in.	in.	in.	in.	in.
	1955					Long-term average				
Foremost	1.33	4.25	0.81	5.54	16.44	0.92	1.67	2.73	1.33	13.26(29)[1]
Claresholm	1.58	4.36	0.95	3.89	18.01	1.43	2.30	3.26	1.78	17.94(27)
Nobleford	2.00	3.93	1.46	2.85	16.29	0.96	2.13	3.23	1.27	15.36(17)
	1956					Long-term average				
Foremost	0.39	1.48	2.46	3.49	16.65	0.90	1.66	2.72	1.41	13.37(30)
Claresholm	0.82	1.01	3.19	3.15	17.51	1.40	2.23	3.26	1.85	17.92(28)
Pincher Creek	1.65	2.76	1.94	3.16	18.01	1.70	2.52	3.98	1.60	20.82(34)

[1] Figures in parentheses indicate number of years of records.

Black Soil Zone (17), replaced Nobleford. Plots were arranged in a randomized block design with four replications. Each treatment consisted of four rows, 20½ feet long, spaced 9 inches apart. The plots were seeded with a power rod-row V-belt seeder with double-disk furrow-openers, which placed the fertilizer in the row with the seed. Chinook wheat was seeded in 1955, and Rescue wheat in 1956, at the rate of 1 bushel per acre at Foremost and at 1¼ bushels per acre at the other locations. In both years all plots, except those at Pincher Creek, were sprayed for weed control with low volatile ester of 2,4-D at 4 oz. acid equivalent per acre when the wheat was in the 4-leaf stage. There were not enough weeds in the plots at Pincher Creek to justify spraying.

The experimental areas had medium to heavy trash covers in both years. At Nobleford in 1955 a blade cultivator and a rod weeder were used to prepare the seedbed, while at the other locations, in both years, the plots were one-way-disked prior to seeding. At Pincher Creek in 1956 the surface soil was dry to a depth of 2½ inches, with moist soil then extending to a depth of 32 inches. At the other locations in both 1955 and 1956 the soil was moist to at least 32 inches at the time of seeding.

Four rates of nitrogen (N at 10, 20, 40, and 80 lb. per acre) and four rates of phosphorus (P_2O_5 at 0, 10, 20, and 40 lb. per acre) were applied in all combinations. A check treatment receiving no fertilizer was included, giving a total of 17 treatments in each test. Nitrogen was supplied in the form of ammonium nitrate (33.5-0-0), and phosphorus in the form of triple superphosphate (0-42-0).

The two centre rows of each plot, trimmed to a length of 16½ feet, were harvested at maturity and threshed, and the yield was calculated in bushels per acre. Nitrogen was determined on ground, oven-dry samples of the grain by the Kjeldahl method (1). Percentage protein on an oven-dry basis was calculated by multiplying the percentage nitrogen by 5.7. The yield and protein data were treated statistically by the analysis of variance, and the 5 per cent level was taken as the level of significance.

RESULTS AND DISCUSSION

During 1955 precipitation was above average at the three experimental locations, and during 1956 it was above average at Foremost but below average at Claresholm and Pincher Creek, as shown in Table 1. The monthly rainfall for April, May, June, and July of both years was variable when compared with long-term averages, but in every case rainfall was lower than average during June and higher than average during July. The low June rainfall had no visible effect on the growth of the wheat in either year.

Data showing the effect of the fertilizer treatments on the yield and protein content of spring wheat seeded on stubble fields are summarized in Table 2.

In 1955 at Foremost nitrogen fertilizer at all rates significantly increased the yield, but additions of phosphorus had no significant effect on the yield. At Nobleford and Claresholm yields were significantly increased by the high rates of nitrogen, and phosphorus additions resulted in further yield increases. Moisture did not seem to be a limiting factor in the production of

TABLE 2.—THE YIELD AND PROTEIN CONTENT[1] OF SPRING WHEAT GROWN ON STUBBLE FIELDS IN SOUTHERN ALBERTA IN 1955 AND 1956 AS AFFECTED BY VARIOUS FERTILIZER TREATMENTS

Treatment[2]		1955						1956					
		FOREMOST (Chin silt loam)		NOBLEFORD (Leth. silt loam)		CLARESHOLM (Carmangay fine sandy loam)		FOREMOST (Chin silt loam)		PINCHER CREEK (Halifax clay)		CLARESHOLM (Carmangay fine sandy loam)	
N	P_2O_5	Yield	Protein	Yield	Protein	Yield	Protein	Yield	Protein	Yield	Protein	Yield	Protein
lb./ac.		bu./ac.	%	bu./ac.	%	bu./ac.	%	bu./ac.	%	bu./ac.	%	bu./ac.	%
0	0	19.7	14.07	20.3	12.54	11.7	13.57	18.7	13.58	24.7	11.75	6.0	14.94
10	0	27.4	14.19	20.6	11.83	19.3	12.53	19.6	13.35	20.4	12.23	7.1	15.00
20	0	26.7	13.99	26.2	11.81	15.2	12.79	23.7	13.19	22.6	12.64	7.0	15.06
40	0	30.7	14.38	30.4	12.39	23.6	14.05	30.2	13.60	20.2	14.25	9.7	15.17
80	0	33.8	15.41	33.4	13.68	22.3	15.99	35.7	15.70	20.7	16.59	10.5	15.23
10	10	24.8	13.71	21.2	10.97	18.9	12.92	20.0	13.19	24.1	12.16	8.5	14.84
20	10	30.2	14.57	31.2	13.34	20.8	12.77	25.5	13.68	25.4	12.80	10.3	14.83
40	10	34.4	14.47	34.7	12.30	23.1	12.54	28.2	14.16	22.4	13.99	9.9	14.68
80	10	32.2	15.86	39.5	14.28	29.9	15.59	29.2	16.51	21.1	16.25	11.2	15.17
10	20	27.4	13.87	25.7	12.59	18.1	12.67	21.3	13.46	22.2	12.02	10.3	14.74
20	20	30.6	14.08	27.7	11.91	24.5	12.58	26.1	13.77	21.1	12.81	12.0	14.57
40	20	31.0	14.14	36.3	12.16	26.4	12.70	31.8	13.99	21.6	14.48	15.4	15.31
80	20	32.9	15.28	38.8	13.85	32.1	14.41	31.4	15.74	17.6	16.45	11.6	15.42
10	40	28.7	13.65	30.0	13.04	16.8	12.03	26.1	15.08	25.8	11.86	12.5	14.40
20	40	27.8	13.77	26.5	11.47	24.5	12.29	25.1	13.28	24.1	12.72	15.2	14.88
40	40	33.1	14.43	36.1	12.78	29.1	12.09	27.4	13.44	19.8	14.14	12.5	15.08
80	40	36.8	15.36	46.0	13.37	37.6	13.67	34.6	15.32	18.5	16.00	12.4	15.22
L.S.D.[3]		5.3	0.64	5.9	1.26	5.0	1.15	7.3	1.24	D.N.S.	0.82	3.2	0.72

[1] Kjeldahl N x 5.7 (oven-dry basis)
[2] N from ammonium nitrate (33.5-0-0) and P_2O_5 from triple superphosphate (0-42-0)
[3] 5% level of significance

wheat on stubble fields in 1955, and there were indications that higher rates of nitrogen, and possibly of phosphorus, would have caused further increases in yield.

Results in 1956 showed significant yield increases at Foremost and Claresholm due to the use of high rates of nitrogen fertilizer, and at Claresholm phosphorus additions to the nitrogen caused further significant yield increases in some cases. In June of 1956 high winds caused soil drifting at Claresholm, which affected the growth of the wheat and possibly the response to fertilizer, so that yields, and increases due to fertilizer treatment, were not as high as in 1955.

At Pincher Creek no significant yield differences resulted from the fertilizer treatments in 1956. The surface soil was dry at the time of seeding (May 25), and stand counts indicated that the high rates of nitrogen fertilizer placed with the seed in this relatively dry soil reduced germination. Rainfall during June at Pincher Creek was lower than average, and, considering this factor along with the dry surface soil, it is possible that moisture could have limited yields.

Significant differences in protein content of spring wheat grown on stubble fields were obtained at each location in both years. At Foremost and Claresholm in 1955 the high rate of application of nitrogen fertilizer caused significant increases in protein content over the unfertilized check. However, low rates of phosphorus along with high rates of nitrogen were necessary before significant increases were obtained at Nobleford. At Foremost and Pincher Creek in 1956 the high rates of nitrogen resulted in increased protein content as compared with the unfertilized check. At Claresholm in 1956 significant differences between treatments were noted, but no treatment was significantly better than the unfertilized check. The soil drifting mentioned previously could have had some effect on the response to fertilizer at this location.

The effect of nitrogen on the protein content of wheat at Claresholm in 1955 was significantly influenced by additions of phosphorus. The addition of 20 and 40 lb. of phosphorus per acre to the 40- and 80-lb. rates of nitrogen resulted in significant reductions in protein content when compared to the nitrogen applications alone. At the other locations, in both 1955 and 1956, no consistent effect due to phosphorus was noted.

Nitrogen at rates of 10 and 20 lb. per acre did not significantly affect the protein content of the wheat in either year, although in most cases non-significant decreases resulted from the application of nitrogen at these rates. Higher protein contents were obtained from applications of 40 lb. of nitrogen per acre at two locations in 1955 and at all three locations in 1956, but the increases were not significant except at Pincher Creek. Nitrogen at the rate of 80 lb. per acre resulted in significant increases in protein content at Foremost and Claresholm in 1955, with the increase at Nobleford approaching significance. In 1956 the increases in protein content due to applications of 80 lb. of nitrogen per acre were significant at Foremost and Pincher Creek but not at Claresholm.

From the results of these experiments it would appear that increased yields of spring wheat grown on stubble fields in southern Alberta may be obtained from the use of nitrogen fertilizers when moisture is adequate.

More than 40 lb. of nitrogen per acre, and at least 80 lb. per acre in some cases, must be applied before increases in protein content of spring wheat grown under these conditions can be expected. The addition of 20 lb. or more of phosphorus per acre to the nitrogen application may reduce the effect of the nitrogen on the protein content.

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GROWTH OF FOUR RACES OF *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY IN SYNTHETIC MEDIA[1]

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ABSTRACT

A basal medium is described containing three inorganic salts, dextrose, asparagine and thiamine, that supported the growth of races 1,2 and 1,2,3 of *Phytophthora infestans*, but not that of races 0 and 1. The medium supported growth of races 0 and 1 when peptone was added, but not when 24 other nitrogen sources were added singly. The addition of any one of 24 carbon sources failed to promote growth of race 1. None of 12 vitamins tested promoted the growth of all four races. However, one of the vitamins, ascorbic acid, and another reducing compound, sodium thioglycollate, promoted growth of races 0 and 1. A number of organic nitrogen compounds were utilized by races 1,2 and 1,2,3 but others, along with ammonium and nitrate salts, were not. Races 1,2 and 1,2,3 differed in their ability to utilize lysine and norleucine, and several nitrogen sources inhibited growth of both races. The best carbon sources for the growth of races 1,2 and 1,2,3 were dextrose, fructose, sucrose and glycerol.

INTRODUCTION

Resistance of the potato to *Phytophthora infestans* (Mont.) de Bary is controlled by at least four independent dominant genes (3), and 16 physiologic races of the fungus can be identified on the basis of differences in their pathogenicity to differential host plants having the various combinations of these genes. But the mechanism whereby the genes confer resistance, and why the races differ in their pathogenicity to the differential hosts, are not understood. A possible explanation for these problems is suggested by studies which indicate that the avirulence of certain biochemical mutants of plant and animal pathogens to specific hosts is caused by the inability of the host to provide the pathogen with the required nutritional environment (1, 2, 7, 8, 9, 10, 12). Although little information is available to show that such an explanation applies to resistance to *P. infestans* in potato, the possibility has been suggested by Pristou and Gallegly (21). With this in mind, the author began a study to determine if differences in the nutritional requirements of four races of the fungus could be demonstrated in synthetic media.

Studies on the nutrition of *Phytophthora* species have shown that, although many compounds can be utilized as carbon sources, individual species and isolates of the same species may differ in their ability to utilize certain compounds (14, 17, 28, 29). Similarly, although a number of organic and to a lesser extent inorganic compounds are utilized as nitrogen sources, species differ in their ability to utilize specific compounds (13, 15, 20, 29). It has also been reported that races 0 and 4 of *P. infestans* differ in their utilization of a number of nitrogen sources, and in their ability to grow in certain concentrations of ammonium salts and succinic acid (6). Thiamine

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is the only vitamin that has been shown to be required by *Phytophthora* species (19, 23). However, growth factors for races 0 and 4 of *P. infestans* have been reported present in raw sugar and yeast extract (6). Recent studies have shown that *P. parasitica* requires the trace elements iron, manganese and copper, and that it has a relatively high zinc requirement (15).

MATERIALS AND GENERAL METHODS

Monosporangial cultures of races 0; 1; 1,2 and 1,2,3 of *P. infestans* were used; races 0 and 1 were obtained from isolates supplied by K. M. Graham, Botany and Plant Pathology Laboratory, Ottawa; races 1,2 and 1,2,3 were from local isolates. The code used for naming races was that proposed by Black, Mastenbroek, Mills and Peterson (4). Stock cultures of the races were maintained in flasks of rye steep liquor (25) incubated at a temperature of 19°–21° C. All nutrient solutions were inoculated with pieces of mycelium of approximately 0.2 mg. dry weight cut aseptically from the perimeter of washed mycelial mats from 10- to 14-day-old stock cultures.

Glassware was cleaned in sulphuric acid-potassium dichromate cleaning mixture and rinsed several times with distilled water. The rinse-water and that used in nutrient solutions was distilled twice—first through a Barnstead still, and then through a glass one.

All inorganic chemicals were of reagent grade; organic chemicals were obtained from the Nutritional Biochemical Company, Cleveland, Ohio. Cultures were grown in 125-ml. Erlenmeyer flasks or 150 mm. x 20 mm. Pyrex test-tubes to which 25 ml. and 10 ml. respectively of solution were added. The pH of the synthetic nutrient solutions was measured with a Beckman Model G pH meter and adjusted to pH 6.3–6.5 with dilute NaOH or HCl before being autoclaved at 240° F. for 10 minutes. Cultures were incubated at 19-21° C. for 3 weeks; the mycelial mats were then removed, pressed between blotters to remove excess liquid, dried to a constant weight at 90° C., and weighed.

EXPERIMENTAL

Basal Medium

Preliminary studies showed that a modification of the synthetic nutrient solution suggested by Payette and Perrault (19) for the culture of *P. infestans* would support growth of races 1,2 and 1,2,3. This modified solution contained all the compounds present in the Payette-Perrault solution with the exception of glycine, ammonium nitrate and manganese sulphate. The solution was further modified by the addition of the amino acids methionine and tryptophane, which were included following studies on the inhibiting effect of certain antimetabolites on the growth of the fungus in rye steep liquor. Although the modified medium supported good growth of both races, it was too complex to be of use in further comparative studies on the growth of races in nutrient solution. Therefore, in an attempt to find a simpler nutrient solution supporting growth of both races, a series of solutions was prepared in which each of the compounds present in the modified

TABLE 1.—EFFECT OF REMOVING A SINGLE COMPONENT FROM A COMPLEX MEDIUM ON THE GROWTH OF RACES 1,2 AND 1,2,3 OF *P. infestans*

Compound removed	Average dry weight of mycelium per flask, milligrams[1]	
	Race 1,2	Race 1,2,3
None[2]	27.0	27.0
Dextrose	0.3	0.4
K_3PO_4	0.3	0.3
$MgSO_4 \cdot 7H_2O$	1.8	1.6
Thiamine HCl	5.5	5.0
Na_2HPO_4	25.8	25.0
Fumaric acid	26.6	25.5
Succinic acid	25.7	24.6
$CaCl_2$	26.0	28.2
dl-Methionine	29.3	24.7
l-Asparagine	23.7	22.5
Ammonium tartrate	25.4	25.3
dl-Tryptophane	28.0	27.3
Boric acid	26.2	27.5
$FeCl_3 \cdot 6H_2O$	29.2	25.1

[1] Differences of 7.4 are significant at the 5 per cent level.
[2] The complete medium contained the following, in gm./litre: dextrose, 5.0; K_3PO_4, 1.0; Na_2HPO_4, 1.0, $MgSO_4 \cdot 7H_2O$, 1.0; fumaric acid, 0.25; succinic acid, 0.25; $CaCl_2$, 0.02; dl-methionine, 1.5; l-asparagine, 1.0; ammonium tartrate, 1.0; dl-tryptophane, 0.5; in p.p.m.: boric acid, 1.0; $FeCl_3 \cdot 6H_2O$, 1.0; thiamine HCl, 0.2.

medium was deleted singly. Two hundred ml. of each solution was prepared and 25 ml. added to each of eight 125-ml. Erlenmeyer flasks. Four flasks of each solution were inoculated with mycelium of race 1,2 and four with mycelium of race 1,2,3. Before use, the mycelial mats from which the inoculum was taken were washed in three changes of physiologic salt solution. This experiment was done three times to make a total of 12 flasks of each treatment inoculated with each race. The results were analysed by the analysis of variance method and are shown in Table 1.

The results show that, with the exception of dextrose, K_3PO_4, $MgSO_4$, and thiamine, none of the compounds tested was essential for growth; and under the conditions employed the two races do not differ in their requirement for any of the 14 compounds tested. It was found that growth of the two races in a nutrient solution containing the four compounds mentioned above, plus 1.0 gm./litre l-asparagine and 1.0 mg./litre ferric chloride, was comparable to that obtained in the modified and the complete Payette-Perrault medium. This simplified solution, referred to hereafter as the basal medium, contained the following: K_3PO_4, 1 gm.; $MgSO_4$, 1 gm.; dextrose, 5 gm.; $FeCl_3$, 1.0 mg.; l-asparagine, 1 gm.; thiamine, 0.2 mg. per litre of double-distilled water.

Growth in Basal Medium and in Rye Steep Liquor

A comparison was made between the growth of races 0; 1; 1,2 and 1,2,3 in basal medium and in rye steep liquor, the natural liquid medium found by Snieszko, Carpenter, Lowe and Jakob (25) to support best growth of the fungus. In this experiment four flasks containing 25 ml. of medium were inoculated with mycelium of the four races that had been washed four times in basal medium. The experiment was repeated three times and the results analysed by the analysis of variance method.

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TABLE 2.—GROWTH OF RACES 0; 1; 1,2 AND 1,2,3 OF *P. infestans* IN A BASAL MEDIUM AND IN RYE STEEP LIQUOR

Race	Growth per flask in milligrams[1]	
	Basal medium	Rye steep liquor
0	0.2	13.1
1	0.2	12.9
1,2	35.6	25.4
1,2,3	37.7	26.8

[1] Differences of 7.3 are significant at the 5 per cent level.

The results (Table 2) show that races 0 and 1 grew in rye steep liquor but not in the basal medium. Races 1,2 and 1,2,3 grew in both media, but growth in the basal medium was significantly better than that in rye steep liquor. Additional experiments showed that the poorer growth of races 1,2 and 1,2,3 obtained in rye steep liquor may have been caused by a lack of nitrogen, and it was found that the addition of 1.0 gm./litre l-asparagine to the medium gave growth equal to that obtained in the basal medium.

The following studies on the nutritional requirements of the four races were undertaken to determine (a) if the inability of races 0 and 1 to grow in the basal medium was due to a specific nitrogen, carbon or vitamin requirement, and (b) if races 1,2 and 1,2,3 and possibly races 0 and 1 differed in their nitrogen, carbon, or vitamin requirements.

Nitrogen Requirements

Nineteen organic and four inorganic compounds were tested to determine (a) their utilization by the four races as sole sources of nitrogen, and (b) their effect on growth when used in combination with asparagine. Each compound was added singly to the basal medium with and without asparagine. In addition, three other organic compounds, Bacteriological peptone, d-alanine and l-alanine, were tested to determine their value as sole sources of nitrogen. Each compound tested, with the exception of the dl-amino acids, was added at a rate sufficient to provide 25 mg. nitrogen per 100 ml. of solution; dl-amino acids were added at twice this rate on the assumption that the d-isomer may not be available to the fungus. The solutions were added to test-tubes at the rate of 10 ml. per tube. Duplicate tubes of each solution were inoculated with mycelium that had been washed in three changes of the basal medium minus asparagine. The results obtained were arranged in five classes on the basis of dry weight (milligrams) of mycelium produced per tube. The classes were as follows: class 0 (0-0.2), class 1 (0.3-4.3), class 11 (4.4-8.4), class 111 (8.5-12.5), class 1111 (12.6-16.6). This experiment was done twice with similar results. Table 3 shows the results obtained with races 1,2 and 1,2,3 in one experiment. Races 0 and 1 grew (class 11) in the solution containing peptone as a source of nitrogen, but did not grow in any of the other solutions tested.

TABLE 3.—GROWTH OF RACES 1,2 AND 1,2,3 OF *P. infestans* IN A SYNTHETIC MEDIUM TO WHICH VARIOUS NITROGEN-CONTAINING COMPOUNDS WERE ADDED SINGLY AND IN COMBINATION WITH ASPARAGINE

Compound added[1]	Amount of growth[2]			
	With asparagine		Without asparagine	
	Race 1,2	Race 1,2,3	Race 1,2	Race 1,2,3
dl-alpha-Alanine	1111	1111	1	1
Beta-Alanine	111	111	0	0
l-Alanine	—	—	1111	1111
d-Alanine	—	—	0	0
Glycine	11	11	1	1
dl-alpha-Amino-n-Butyric acid	1	1	1	1
l-Arginine HCl	111	111	1	1
l-Asparagine	1111	1111	1111	1111
l-Aspartic acid	111	111	11	11
l-Cystine	1	1	0	0
l-Glutamic acid	1111	1111	11	11
l-Glutamine	1111	1111	1	1
l-Histidine HCl	1111	1111	1	1
dl-Norleucine	1	0	1	0
l-Lysine HCl	1111	1111	1	0
l-Phenylalanine	1	1	1	1
l-Proline	1111	1111	1	1
l-Serine	111	111	1	1
l-Threonine	11	11	1	1
l-Tyrosine	1	1	1	1
l-Valine	1	1	1	1
Peptone	—	—	1111	1111
Ammonium tartrate	1	1	1	1
Ammonium sulphate	111	111	1	1
Ammonium nitrate	11	11	1	1
Potassium nitrate	111	111	0	0
None	1111	1111	0	0

[1] All compounds, except dl-amino acids, were added at a rate sufficient to give 25 mg. N/100 ml. of solution; dl-amino acids were added at twice this rate.

[2] 0 = 0 – 0.2 mg., 1 = 0.3 mg. – 4.3 mg., 11 = 4.4 mg. – 8.4 mg., 111 = 8.5 mg. – 12.5 mg., 1111 = 12.6 mg. – 16.6 mg.

The best sources of nitrogen for races 1,2 and 1,2,3 were asparagine peptone, and l-alanine. However, d-alanine was not utilized and the two races therefore behaved similarly to *P. parasitica* (5) in their utilization of the two isomers of alanine. Moderate growth of both races occurred when either aspartic or glutamic acid was the only nitrogen source. In this and subsequent experiments it was shown that ammonium and nitrate salts were poor sources of nitrogen for races 1,2 and 1,2,3. A number of compounds had a marked inhibiting effect on growth of the two races when added with asparagine; these included glycine, alpha-amino-n-butyric acid, cystine, phenylalanine, threonine, tyrosine, valine, ammonium tartrate and ammonium nitrate. Norleucine also inhibited the growth of both races, but the inhibiting effect on race 1,2,3 was more pronounced than that on race 1,2. Also, apparently there is a difference in the ability of the two races to utilize norleucine and lysine as single nitrogen sources. Numerous subsequent tests showed that the races can be distinguished on the basis of their utilization of either norleucine or lysine as a sole source of nitrogen, and on their ability to grow in the basal medium in the presence of norleucine.

Carbon Requirements

Twenty-five compounds were tested as sources of carbon for races 1; 1,2 and 1,2,3. Each compound was substituted singly for dextrose in the basal medium in amounts sufficient to give a concentration of 400 mg. carbon per 100 ml. of solution. Solutions were prepared in flasks in triplicate and inoculated with mycelium that had been washed three times in basal medium minus dextrose, left 16 hours in the third washing, and washed a fourth time before use. Each compound was tested twice with similar results. As in the previous experiment, the results obtained in each experiment were arranged in classes on the basis of weight (milligrams) of mycelium produced per flask. The classes were as follows: class 0 (0–0.2), class 1 (0.3–16.3), class 11 (16.4-32.4), class 111 (32.5-48.5), class 1111 (48.6-64.6). Table 4 shows the results of one experiment.

Race 1 did not grow in any solution and apparently its inability to grow in the basal medium was not due solely to a specific requirement for any one of the carbon sources tested. The best sources of carbon for races 1,2 and 1,2,3 were dextrose, fructose, sucrose and glycerol; mannose was also utilized to some extent. Some growth of races 1,2 and 1,2,3 occurred in the control flasks, probably because of the utilization of asparagine as a

TABLE 4.—GROWTH OF RACES 1; 1,2 AND 1,2,3 OF *P. infestans* IN A SYNTHETIC MEDIUM TO WHICH VARIOUS COMPOUNDS WERE ADDED AS SINGLE SOURCES OF CARBON

Compound added[1]	Amount of growth[2]	
	Race 1,2	Race 1,2,3
Arabinose	1	1
Xylose	1	1
Mannose	11	11
Dextrose	1111	1111
Galactose	1	1
Fructose	1111	1111
Lactose	1	1
Maltose	1	1
Cellobiose	1	1
Sucrose	111	111
Raffinose	1	1
Starch	1	1
Dextrin	1	1
alpha-Methyl glucoside	1	1
Adonitol	0	0
Mannitol	0	0
Dulcitol	0	0
Sorbitol	0	0
Glycerol	111	111
Acetic acid	0	0
Succinic acid	0	0
Tartaric acid	0	0
Oxalic acid	0	0
Citric acid	0	0
Malic acid	0	0
Control (asparagine)	1	1

[1] Compounds were added at a rate sufficient to give 400 mg. C/100 ml. of solution.

[2] 0 = 0 – 0.2 mg., 1 = 0.3 mg. – 16.3 mg., 11 = 16.4 mg. – 32.4 mg., 111 = 32.5 mg. – 48.5 mg., 1111 = 48.6 mg. – 64.6 mg. Race 1 did not grow in any solution tested.

source of both nitrogen and carbon. Organic acids and the alcohols, except glycerol, were not utilized, but appeared to inhibit growth at the concentrations used. None of the remaining 10 compounds tested promoted growth of races 1,2 and 1,2,3 in excess of that obtained in the control.

Vitamin Requirements

As races 0 and 1 grew in the basal medium only on the addition of peptone, which has been reported to contain most of the water-soluble vitamins (26), an experiment was made to determine if the races were deficient for any of 12 vitamins. Races 1,2 and 1,2,3 were also included in the experiment. The vitamins tested were: biotin, (0.05 p.p.m.); folic acid, (100 p.p.m.); pyridoxine, (2 p.p.m.); nicotinic acid, (2 p.p.m.); riboflavin, (1 p.p.m.); ascorbic acid, (160 p.p.m.); nicotinamide, (2 p.p.m.); choline chloride, (200 p.p.m.); p-amino-benzoic acid, (3 p.p.m.); calcium pantothenate, (2 p.p.m.); inositol, (10 p.p.m.); and vitamin B_{12} (0.05 p.p.m.). Each vitamin was added separately to the basal medium and mycelium of each race was inoculated to three tubes of each solution. The mycelium was washed three times in basal medium, left for 16 hours in the third washing, and washed a fourth time before using. The experiment was done twice with similar results.

None of the vitamins tested stimulated the growth of races 1,2 and 1,2,3 at the concentrations used, and apparently these races are not deficient for any one of the 12 vitamins. The results showed, however, that growth of races 0 and 1 occurred only in the solution containing ascorbic acid, and suggested that this vitamin is required by both races. But, in view of the fact that no fungus has been definitely shown to be deficient for this vitamin (11), such a conclusion was questioned and another explanation for the results sought.

Since ascorbic acid is a strong reducing agent, the growth of races 0 and 1 obtained in the basal medium upon addition of the vitamin may have resulted from an increase in the reducing properties of the medium. An experiment, therefore, was made to determine if other reducing agents would give results similar to that obtained with ascorbic acid. The reducing compounds tested (glutathione, l-cysteine HCl and sodium thioglycollate) were added to the basal medium at a concentration of 160 mg. per litre; and four flasks of each solution were inoculated with mycelium of race 1 which had been washed in the basal medium as described in the above experiment on vitamin requirement. The results of this experiment

TABLE 5.—EFFECT OF REDUCING COMPOUNDS ON THE GROWTH OF RACE 1 OF *P. infestans* IN A BASAL MEDIUM

Compound added (160 mg./litre)	Growth per flask, milligrams
Glutathione	0
l-Cysteine HCl	0
Sodium thioglycollate	68
Ascorbic acid	55
None	0

(Table 5) show that the addition of sodium thioglycollate to the synthetic medium promoted growth of race 1 comparable to that obtained with ascorbic acid. Cysteine and glutathione had no apparent effect on growth. Similar results were obtained with race 0.

DISCUSSION

The greatest difference between races was found in their ability to grow in the basal medium. The failure of races 0 and 1 to grow in this medium, however, was apparently not due solely to a deficiency for any one of the various amino acids or vitamins tested, or to a requirement for a specific nitrogen or carbon source. French (6) found that race 0 was unable to utilize asparagine or aspartic acid as a nitrogen source, but obtained growth of this race with peptone and with a mixture of five amino acids. The good growth of races 0 and 1 obtained in the basal medium when ascorbic acid was added shows that these two races were able to utilize asparagine and did not require a complex nitrogen source.

Ascorbic acid has previously been reported to stimulate growth of *P. infestans* (20), but it has not been definitely shown to be a requirement of any fungus (11). Virtanen and Hausen (27) found that, to obtain normal growth of wheat and pea seedlings in synthetic media when nitrate was used as a source of nitrogen, a high concentration of ascorbic acid was required; but the vitamin was not required when ammonium sulphate was used. Other reducing compounds had an effect similar to that of ascorbic acid and the authors suggest that the observed effect of the vitamin is due to its reducing properties. Ascorbic acid and thioglycollic acid also restore the activity of enzymes inactivated by oxidization of their sulfhydryl groups (24). The fact that the reducing compound sodium thioglycollate promoted growth of races 0 and 1 equal to that obtained with ascorbic acid suggests that the observed effect of the vitamin on the growth of these races may be due to its reducing properties. However, failure of the races to grow when the reducing compounds glutathione and cysteine were used indicates that further investigations are required.

The results of studies on the sources of nitrogen utilized by races 1,2 and 1,2,3 show that these two races require an organic source of nitrogen and, therefore, belong to group IV of Robbins classification (22). The ammonium and nitrate salts tested were poorly utilized and when added to the basal medium appeared to inhibit growth; this inhibiting effect was particularly notable when ammonium tartrate was added. Yet ammonium tartrate did not appear to inhibit growth when used along with the compounds shown in Table 1, so its inhibiting effect presumably depends upon the composition of the medium. This is possibly why Peterson (20) found ammonium sulphate to be a better source of nitrogen for *P. infestans* than asparagine or potassium nitrate. Recently it has been shown that growth of races 0 and 4 is inhibited by ammonium sulphate (6); but race 0 is inhibited by a much lower concentration than race 4. No difference was noted in the inhibiting effect of ammonium or nitrate salts on the growth of races 1,2 and 1,2,3. Yet the two races did appear to differ in their utilization of norleucine and lysine as sources of nitrogen, and in their

ability to grow in the basal medium when norleucine was added. When glycine and dextrose are autoclaved together a substance is produced which inhibits growth of a number of *Phytophthora* spp. (16). The inhibition effect on growth of races of *P. infestans* noted when certain nitrogen sources were added to the basal medium may also be caused by the formation of some toxic principle; and differences noted between the races may be the result of a difference in their sensitivity to the toxic principle.

According to Black each potato race of *P. infestans* can be considered as a group or population the members of which, although alike in their pathogenicity to the major gene differential hosts, can be expected to show certain minor differences (3). Such differences have been demonstrated on potato tubers (3, 18) and tomato plants (30). This suggests that a study of the nutrition of a number of isolates of the same race should be made before the differences that have been demonstrated between single isolates are accepted as being characteristic of the races concerned.

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THE APPLICATION OF CHROMATOGRAPHIC METHODS TO A STUDY OF THE SUSCEPTIBILITY OF SOYBEAN TO STEM CANKER[1]

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ABSTRACT

Chromatographic methods were used in an attempt to discover why soybeans, which are highly susceptible to infection by *D. phaseolorum* var. *caulivora* earlier in their development, become less susceptible as they grow older. Eighteen amino compounds in hydrolyzates of bark of soybean stems were identified by co-chromatography with substances of known constitution. An increase in concentration of each compound was noted as stem tissue matured and became more resistant to infection. Total nitrogen of the tissues studied also increased with plant maturity.

INTRODUCTION

In artificial inoculation experiments carried out in 1954, Hildebrand (10) found that soybeans, variety Lincoln, which are highly susceptible to infection by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell at midstage in their development, become progressively less so as they approach maturity. As a phase of the present studies Hildebrand's experiments were repeated in as exact detail as possible with the result that his findings were fully confirmed (Table 1). In similar tests recently completed in Minnesota, Frosheiser (8) found that stem canker caused a loss in yield of 10.1 bushels per acre when Blackhawk soybean plants 70 days old were inoculated with mycelium of the pathogen whereas no significant reduction in yield resulted when 84-day-old plants were similarly inoculated. Thus, for stem canker, the phenomenon of a diminishing severity of attack with increasing age of plants seems to have been fairly firmly established. Little is known, however, about the nature of the change that presumably takes place in soybean plants and renders them less susceptible to attack by the pathogen in question. The present writers incline to the view that the change is of a biochemical nature. Instances are not lacking in which naturally synthesized chemical compounds have been found to be associated with resistance to disease induced by micro-organisms (1, 9, 11). For the detection and identification of such chemicals new and far more accurate techniques have largely replaced older chemical methods. Evidence of the wide adaptability and value of chromatographic techniques, for example, can readily be found (6, 7, 12, 13, 14, 16, 17). In the light of these various considerations the present authors chose arbitrarily to explore the possibility of a biochemical change to explain the host-parasite relations outlined above, and in so doing to make use of certain chromatographic techniques. The study was directed chiefly towards protein analysis because of the reputedly high protein content of the soybean plant (15).

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TABLE 1.—EFFECT OF INOCULATION OF SOYBEAN PLANTS, VARIETY LINCOLN, OF THREE AGE GROUPS, WITH ISOLATES OF *D. phaseolorum* VAR. *caulivora*, AS INDICATED BY THE DIFFERENCE BETWEEN THE MEANS OF THE YIELD OF INOCULATED AND NON-INOCULATED PLANTS

Year	Age of plants (days)	Isolate designation	Yield increase in favour of non-inoculated plants, %	Mean difference in favour of non-inoculated plants, gm.	Degrees of freedom	Observed value of t
1954	62	6	100.0	29.15[1]	23	
		7	100.0	33.98	24	
		8	96.1	32.67	24	
		9	95.7	33.69	23	
		10	94.0	33.09	23	
	76	6	42.1	15.83	23	5.38**
		7	33.4	12.43	24	3.74**
		8	53.9	20.06	24	6.96**
		9	9.9	3.84	21	2.46*
		10	34.8	12.70	23	5.59**
	91	6	17.5	5.27	24	2.80**
		7	11.5	3.49	23	2.28*
		8	14.1	4.36	23	3.51**
		9	0.7	1.43	22	1.15
		10	8.4	2.33	24	1.18
	57	6	61.7	17.24	24	10.51**
		10	58.0	17.57	19	9.82**
1956	72	6	12.3	3.76	30	2.56*
		10	17.8	5.41	40	4.03**
	87	6	4.9	1.205	48	.97
		10	4.5	1.157	49	.75

[1]Because almost all of the 62-day-old inoculated plants had been killed, there was virtually no yield from them; thus the difference shown may be regarded as significant without recourse to analysis.
*, **Indicate values of t exceeding the 5 and the 1 per cent level of significance, respectively.

METHODS

The general procedure of direct estimation by comparative colorimetry of amino acids on paper chromatograms employing a Photovolt densitometer was utilized (2, 3, 4, 5).

In the final analysis a quantitative estimation of the amino acids in hydrolyzates of green stem tissue of soybeans, variety Lincoln, was made on two-dimensional chromatograms. The method of sampling approached total sampling since large numbers of specimens were obtained from the experimental plots on July 25, August 9, and August 24. The samples consisted of 5-inch portions cut from the lower half of the stems. All samples were transferred immediately to a deep-freeze unit where they remained until removed for analysis.

Preparation of Extracts

Acid hydrolysis of 25-gram composite samples of fresh (quick-frozen) plant material was carried out in 20 ml. 6N HCl at 108.5° C., ± 1°, under reflux for 24 hours. Each hydrolyzate was evaporated to dryness on a

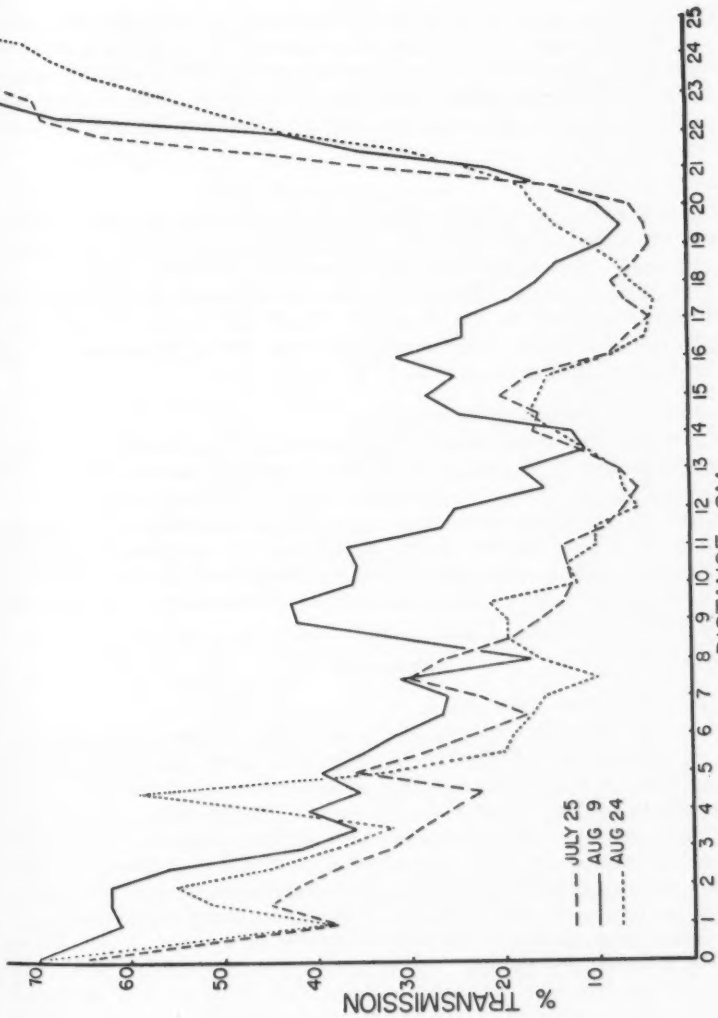

FIGURE 1. Comparison of calibration curves each derived from chromatograms of extracted juice of soybean plants of three different age groups.

steam bath, dissolved in 25 ml. H_2O, filtered, again evaporated to dryness, then dissolved in 5 ml. of 10% aqueous isopropyl alcohol, and stored at room temperature.

Chromatographic Extracts

A 5-lambda aliquot of undiluted hydrolyzates was applied to a spot 2.5 cm. from each edge in the bottom-left corner of a 23 × 28.5 cm. sheet of Whatman No. 1 filter paper. The ascending method was used, the equipment including cylindrical glass chambers 24 in. high × 12 in. in diameter fitted with cover, and four containers, also of glass, 2 in. deep × 4 in. in diameter.

Solvents

The first solvent was liquid phenol: water (100:20 v/v) with beakers of 10 ml. 1% KCN and of 25 ml. 3% NH_4OH also placed in the chromatogram chamber. The second solvent was lutidene:collidine:water (1:1:1 v/v) ± 1% diethylamine. Beakers of 5 ml. 1% KCN and of 5 ml. aqueous phenol also were placed in the chamber. Each dish in the chamber contained 25 ml. of solvent. Chromatograms were left in the solvent for 18 hours at a constant temperature of 21° C. ± 1°.

Development and Reading of Papers

The papers were dried in an air-stream at room temperature, developed with 0.25% ninhydrin in acetone, again dried, and then viewed and warmed on a frosted glass plate over transmitted light. Coloured areas were outlined with pencil and their percentage transmission determined immediately by means of a densitometer and a Klett filter of wave-length 565-630 mμ. Chromatograms were replicated four times both for the standard known amino acids and for the unknowns in the samples of the soybean hydrolyzates.

RESULTS

Analysis of Expressed Plant Juice

The analytical difficulties arising from having to use hydrolyzates of the plant proteins necessitated preliminary trials with cell-free undiluted expressed juice. These first one-dimensional chromatograms were run in phenol solvent and developed with ninhydrin. They showed a great many ninhydrin positive amino compounds which not only frequently overlapped one another but also generally could not be identified even though various dilutions and concentrations of the spotting solution were used. Nevertheless, this preliminary work showed qualitative and quantitative differences in the amino compounds in the expressed juice of soybean plants within the three different age groups. By means of the densitometer the transmission of light through the various intensities of colour was measured, and the measurements were used to plot the calibration curves shown in Figure 1.

Figure 1 shows that the curves derived from chromatograms of plant material of July 25 and of August 24 for the most part correspond closely to one another. In contrast, the curve for August 9 differs widely from the other two, divergences in transmitted light of an order exceeding 22 per

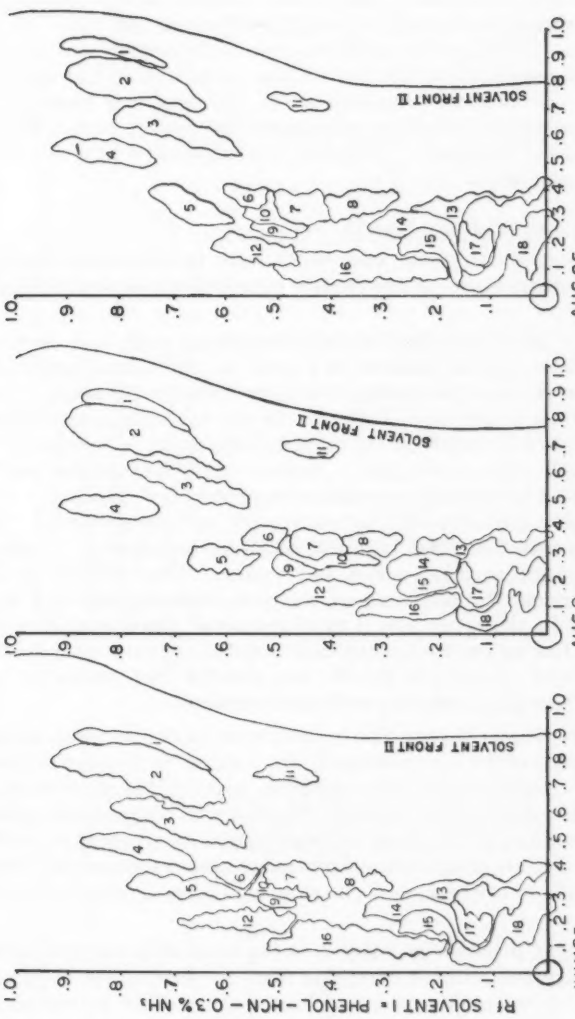

FIGURE 2. Maps of amino compounds in hydrolyzates of soybeans at three developmental stages. See identification of numbers in Table 2, page 161.

cent being indicated. In general, this curve denotes lower concentrations of amino compounds than do the other two. The analytic methods employed showed further that the plant material of August 9 differed from that of July 25 and of August 24, qualitatively as well as quantitatively. On chromatograms of August 9 plant material amino compounds appeared that were not present on those of July 25th and August 24th plant material, and vice versa. Identification of these amino compounds was not forthcoming from the methods employed. These quantitative and qualitative differences in the chemical composition of the host are approximately coincident in time with a decrease in susceptibility on the part of the latter to the stem canker pathogen. Certainly, an association between the two phenomena is suggested.

Analysis of Hydrolyzed Plant Protein

Hydrolyzates of composite samples of fresh (quick-frozen) bark from soybeans of the three different age groups were obtained as described earlier in this paper.

Tracings of the chromatogram outlining amino acids and derivatives in the hydrolyzates are reproduced in Figure 2. Rf values in the phenol solvent and the per cent transmission of light through the spots at wavelength 565-630 mμ are shown in Table 2. In this table the concentration of 18 amino compounds found in the soybean material is compared with μM conc. of each amino compound. Amino compounds of relatively high concentration (0-15 per cent transmission) were leucine + isoleucine, valine, alanine, threonine, glutamic acid, serine, glycine, and aspartic acid. Those of medium concentration (16-35 per cent transmission) were phenylalanine, methionine, tyrosine, arginine, lysine, and cystine. Those of low concentration (36-100 per cent transmission) were proline, hydroxyproline, β alanine and histidine. In the latter group unidimensional chromatograms of the hydrolyzates utilizing butanol-acetic-acid solvent, isatin colour developer and a Klett filter of wavelength 485-550 mμ, showed that proline was present in high concentration relative to hydroxyproline.

Table 2 also shows that in the hydrolyzates of the soybean material, the concentrations of 10 amino compounds, leucine + isoleucine, proline, hydroxyproline, alanine, threonine, arginine, glycine, lysine, cystine, and aspartic acid, are greater than 10 μM. Four amino compounds, valine, β alanine, histidine, and serine, have a concentration about equal to a 10 μM solution of the known compound. Four other amino compounds, phenylalanine, methionine, tyrosine, and glutamic acid have a concentration less than 10 μM.

Of interest, as is shown in Table 2, is the increase in concentration of each amino compound without exception from the first to the third period of sampling, that is, from a period of host susceptibility to the soybean pathogen to one of host resistance to the organism.

A simple Kjeldahl analysis of the hydrolyzates of the three periods showed a slight increase in their nitrogen content. Determinations of nitrogen were 1.61, 1.61, and 1.82 mg./ml. for July 25, August 9, and August 24, respectively.

TABLE 2.—COMPARISON OF AMINO ACIDS AND DERIVATIVES (RF VALUES AND PERCENTAGE TRANSMISSION) IN HYDROLYZATES OF SOYBEAN AT THREE DEVELOPMENTAL STAGES

No.	Compound	Ninhydrin colour	Rf values in phenol solvent				% transmission at wavelength 565-630 mμ			
			10 μM conc.	July 25	Aug. 9	Aug. 24	10 μM conc.	July 25	Aug. 9	Aug. 24
1	Phenylalanine	Brown	.79	.80	.83	.83	14	27	24	20
2	Leucine and isoleucine	Purple	.78	.79	.82	.82	10	9	7	6
3	Valine	Purple	.70	.74	.72	.72	15	15	8	8
4	Proline	Yellow	.87	.83	.87	.87	81	75	70	59
5	Methionine	Purple	.69	.70	.64	.67	15	33	28	22
6	Hydroxyproline	Yellow brown	.53	.57	.53	.53	68	46	34	26
7	α alanine	Purple	.45	.48	.45	.45	14	5	4	4
8	Threonine	Purple	.36	.36	.34	.35	21	13	13	10
9	β alanine	Blue fading	.52	.50	.49	.50	50	52	42	39
10	Histidine	Brownish	.50	.54	.48	.52	40	40	33	26
11	Tyrosine	Brown	.42	.48	.45	.46	24	35	40	25
12	Arginine	Purple	.52	.56	.49	.55	35	27	22	19
13	Glutamic acid	Purple	.19	.20	.19	.19	7	9	9	4
14	Serine	Brown	.25	.27	.27	.27	12	12	8	6
15	Glycine	Blue purple	.29	.24	.25	.23	20	3	3	3
16	Lysine	Purple blue	.37	.35	.27	.35	36	28	22	14
17	Cystine	Red purple	.18	.14	.13	.13	25	21	20	13
18	Aspartic acid	Blue	.07	.06	.10	.09	16	8	6	3

DISCUSSION

At the outset of the present investigations, there seemed reasons for believing that the well-established decrease in susceptibility of soybeans to *D. phaseolorum* var. *caulivora* (Table 1), with increasing age of plants and under conditions of artificial inoculation, might be ascribable to a change of a biochemical nature within the plant. Evidently, as adjudged from the foregoing results, the supposition has some basis in fact; for the diminishing severity of attack by the pathogen has been shown to be associated with a biochemical change in the juice acquired from plants at the mid-period (August 9) of sampling. This change, as indicated by the curve for the analyses of August 9th material is quite different from those derived from the corresponding analyses of July 25th and August 24th materials, respectively. The seeming similarity in the analytical results for the first and third periods of sampling does not necessarily mean that by August 24 24 plants have reverted to a chemical condition similar to that of July 25. Equally possible as the plants approach maturity may be a transformation qualitatively in the ninhydrin-positive compounds present in the plant juices. Standard techniques applied to hydrolyzates of representative plant material showed additional evidence of a biochemical change within the period of experimentation. As Table 2 shows clearly, there was a definite tendency for amino acids to increase in concentration as the plants approached closer to maturity. Thus, by analysis both of fresh and of hydrolyzed plant juice an association has been indicated between a qualitative and quantitative change in the amino acid composition of the plant and a change in host-parasite relations.

It is realized that the methods used in the present studies have their limitations. For example, it is immediately apparent that even an exacting chromatographic analysis of plant material during host-parasite associations may fail to demonstrate that the identifiable compounds are the ones that are utilized by the pathogen during infection and colonization of the host. Despite its limitations the authors feel, however, that their approach to the problem under consideration is a good one, and that a similar approach, making use of the latest and best chromatographic techniques, may provide information that may further help to explain the nature of the biological changes encountered in the study of host-parasite relationships.

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CONVERSION OF LATENT EVAPORATION TO POTENTIAL EVAPOTRANSPIRATION[1]

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ABSTRACT

Potential evapotranspirometers and various evaporation pans are commonly used to estimate potential evapotranspiration. These instruments are expensive, large, difficult to install and use. A black Bellani plate atmometer is suggested as a simple instrument to replace these large tanks. It consists of a black, porous, ceramic plate, mounted on a glazed ceramic cup. The plate surface is kept moist by the water held in the cup. Evaporation from this surface is a measure of the drying ability of the air and is called "latent evaporation". Comparisons of various evaporimeters have shown the Bellani atmometer to be accurate and responsive to meteorological variables.

Latent evaporation was compared with open-pan evaporation (from a 4-foot diameter buried tank) at several sites across Canada. Latent evaporation was also compared with evapotranspiration from irrigated field plots and evapotranspirometer tanks. The conversion factor of 0.0034 inches of evapotranspiration from irrigated fields, and 0.0032 inches of open-pan evaporation for each cubic centimetre of latent evaporation has been tentatively established. Latent evaporation and moisture block methods have shown excellent agreement in scheduling irrigation.

INTRODUCTION

The usefulness of evaporation data is recognized by people in many fields of endeavour. The agronomist's interest arises from the fact that from these data, potential evapotranspiration (total possible evaporation from soil and plants) can be estimated. Information on potential evapotranspiration is useful in soil moisture and irrigation control.

The possibility of using evaporation data in the control of irrigation is dependent on the conclusion that the rate of evaporation of water from soil covered by green vegetation cannot pass a well defined maximum however liberally the vegetation is supplied with water. This maximum rate depends almost entirely on the meteorological conditions and scarcely at all on the nature of the vegetation as long as it is in a state of growth (e.g. green) and completely covers the soil. Penman and Schofield (6) reached this conclusion from the physics of evaporation before there was much direct experimental evidence to support it.

The empirical approach to the estimation of potential evapotranspiration has theoretical and practical disadvantages but has received attention by many workers. Penman's equation for determining evaporation using meteorological parameters is widely used in England in irrigation control (5). Thornthwaite's formula was devised to estimate potential evapotranspiration from temperature and daylength data (10). Blaney also has a formula that has been useful in irrigation control (1). Direct soil sampling is not practical because of the large number of samples necessary for statistical

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reliability. Heat budget and aerodynamic methods (2), while being valuable research tools, do not lend themselves to wide use.

Evaporimeters of various types are widely used to estimate potential evapotranspiration. The open-pan types are bulky, costly and awkward. Spherical or plate ceramic evaporimeters, however, are more economical and convenient to use. The black Bellani plate evaporimeter described by Robertson (7) is structurally more suitable than spherical types and has been found to be responsive, accurate, and easy to use. Robertson and Holmes (9) used this instrument in preliminary work in irrigation planning.

This paper presents further information on the black Bellani plate atmometer and describes the conversion of evaporation from the Bellani plate (henceforth referred to as latent evaporation) to evapotranspiration from irrigated field plots, evapotranspirometers, and evaporation from open pans.

METHODS AND MATERIALS

Response of Various Evaporimeters to Daily Variations in Meteorological Factors

During the summer of 1954 (May 1 to September 30), evaporation from the black Bellani plate, Summerland tank, 4-foot buried tank, white Bellani plate and the Piché evaporimeter was compared, through linear correlation, with several daily meteorological factors. These comparisons are listed

TABLE 1.—RELATIVE RESPONSE OF VARIOUS EVAPORIMETERS TO THE DAILY VARIATION IN THE METEOROLOGICAL FACTORS[1]

Linear Correlation Coefficients

Meteorological Factor	Evaporimeter		
	Black Bellani plate	Summerland tank	4-foot tank
Mean daily temperature	0.460	0.421	0.304
Average wind speed	0.269	−0.012	0.232
Total solar energy	0.771	0.603	0.601
Vapour pressure deficit	0.719	0.695	0.457

Difference from zero required for significance at the .05 level of probability = 0.159; .01 level = 0.208
[1] Observations taken from May 1 to September 30, 1954, N =153

TABLE 2.—RELATIVE RESPONSE OF VARIOUS EVAPORIMETERS TO THE DAILY VARIATION IN METEOROLOGICAL FACTORS[1]

Linear Correlation Coefficients

Meteorological Factor	Evaporimeter		
	Black Bellani plate	White Bellani plate	Piché
Mean daily temperature	0.622	0.508	0.496
Average wind speed	0.231	0.075	0.185
Total solar energy	0.731	0.699	0.622
Average vapour pressure deficit	0.806	0.754	0.491

Difference from zero required for significance at the .05 level of probability =0.361; .01 level =0.463
[1] Observations taken from September 1 − 30, 1954, N =30

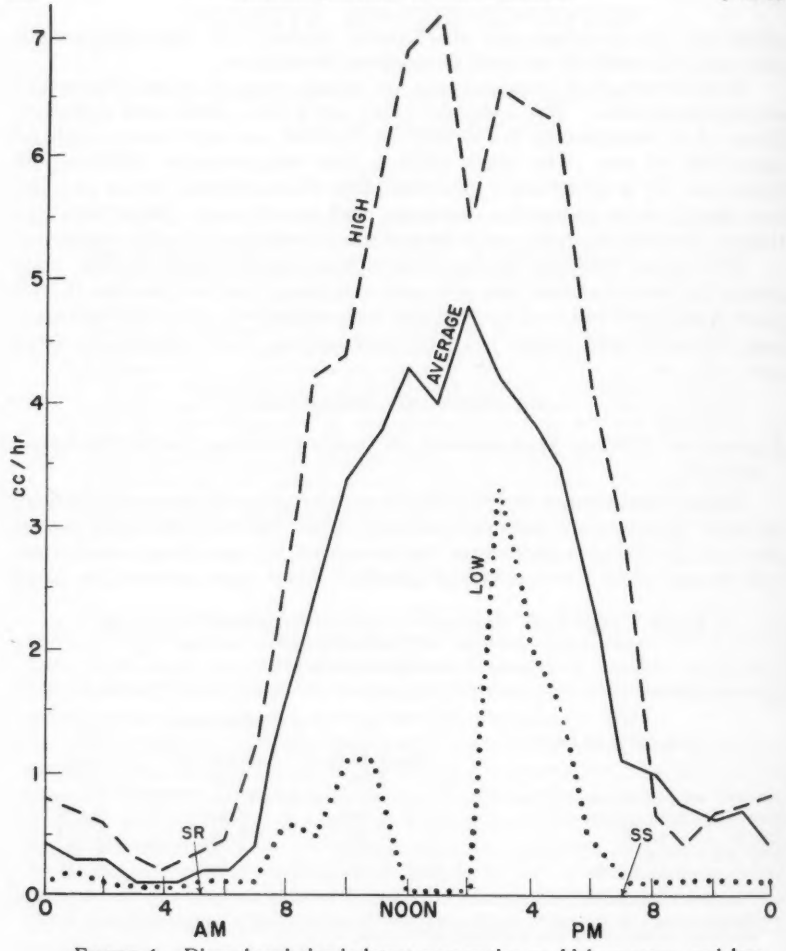

FIGURE 1. Diurnal variation in latent evaporation on high, average, and low evaporation days.

in Tables 1 and 2. The above evaporimeters have been described by Robertson (7). The diurnal variation in latent evaporation (from black Bellani plate) was measured hourly, according to Robertson (7), over several types of drying days. These data are plotted in Figure 1.

Comparison of Open-pan and Latent Evaporation

Measurements of open-pan (in inches) and latent (in cubic centimetres) evaporation were taken during the summer months at Experimental Farms across Canada at the following points: Ottawa, Ont., in 1953, 1954, 1955, and 1956; Swift Current, Sask., Beaverlodge, Alta., and Vauxhall, Alta., in 1954, 1955, and 1956; Kapuskasing, Ont., in 1955, and 1956; and Summerland, B.C., in 1956, according to Robertson's method (8). The monthly

values (69 cases) are compared through linear correlation in Figure 2. The data from Beaverlodge used in the calculations are from a new site established in 1955. Because of the unique exposure of an older site, data were not used but are placed on the graph for comparison.

Comparison of Latent Evaporation and Evaporation from Evapotranspirometers

Measurements of latent evaporation and potential evapotranspiration from evapotranspirometers were made at Swift Current, Sask., and Kapuskasing, Ont., according to the method of Thornthwaite (3). These data are compared in Tables 3 and 4.

Comparison of Latent Evaporation and Moisture Block Methods in Scheduling Irrigation

A comparison of latent evaporation and moisture block methods in scheduling irrigation was carried on at Saanichton, B.C., and at L'Assomption, Que., in 1956. The method used was based on a preliminary Ottawa experiment and is described by Robertson (8). A ratio of 0.0034 inches of evapotranspiration for each cubic centimetre of latent evaporation was used at Saanichton and L'Assomption, and these data are presented in Table 5 and Figure 3 respectively. Rainfall is also shown in Figure 3.

TABLE 3.—RATIO BETWEEN OPEN-PAN AND LATENT EVAPORATION AND POTENTIAL EVAPOTRANSPIRATION AT VARIOUS STAGES OF GROWTH OF WHEAT (SWIFT CURRENT, 1955)

Period and crop stage of growth	O.P./L.E.	P.E.*/L.E.
27 June – 4 July (shot blade)	.0031	.0039
14 July – 17 July (heading)	.0027	.0055
18 July – 21 July	.0026	.0058
22 July – 25 July	.0032	.0061
26 July – 28 July	.0032	.0052
29 July – 1 August	.0031	.0041
2 August – 4 August (filling)	.0033	.0047
5 August – 8 August	.0030	.0032
9 August – 11 August	.0031	.0033
12 August – 15 August (ripe)	.0031	.0029
Average	.0031	.0044

* Small border crop around tanks

TABLE 4.—RATIO BETWEEN POTENTIAL EVAPOTRANSPIRATION AND LATENT EVAPORATION (KAPUSKASING, 1955)

Month	1955*	1956
June	.0036 (18 days)	—
July	.0034 (17 days)	.0035
August	.0037 (18 days)	.0037
September	.0030 (8 days)	—
Average	.0035	.0036

* Rainless days

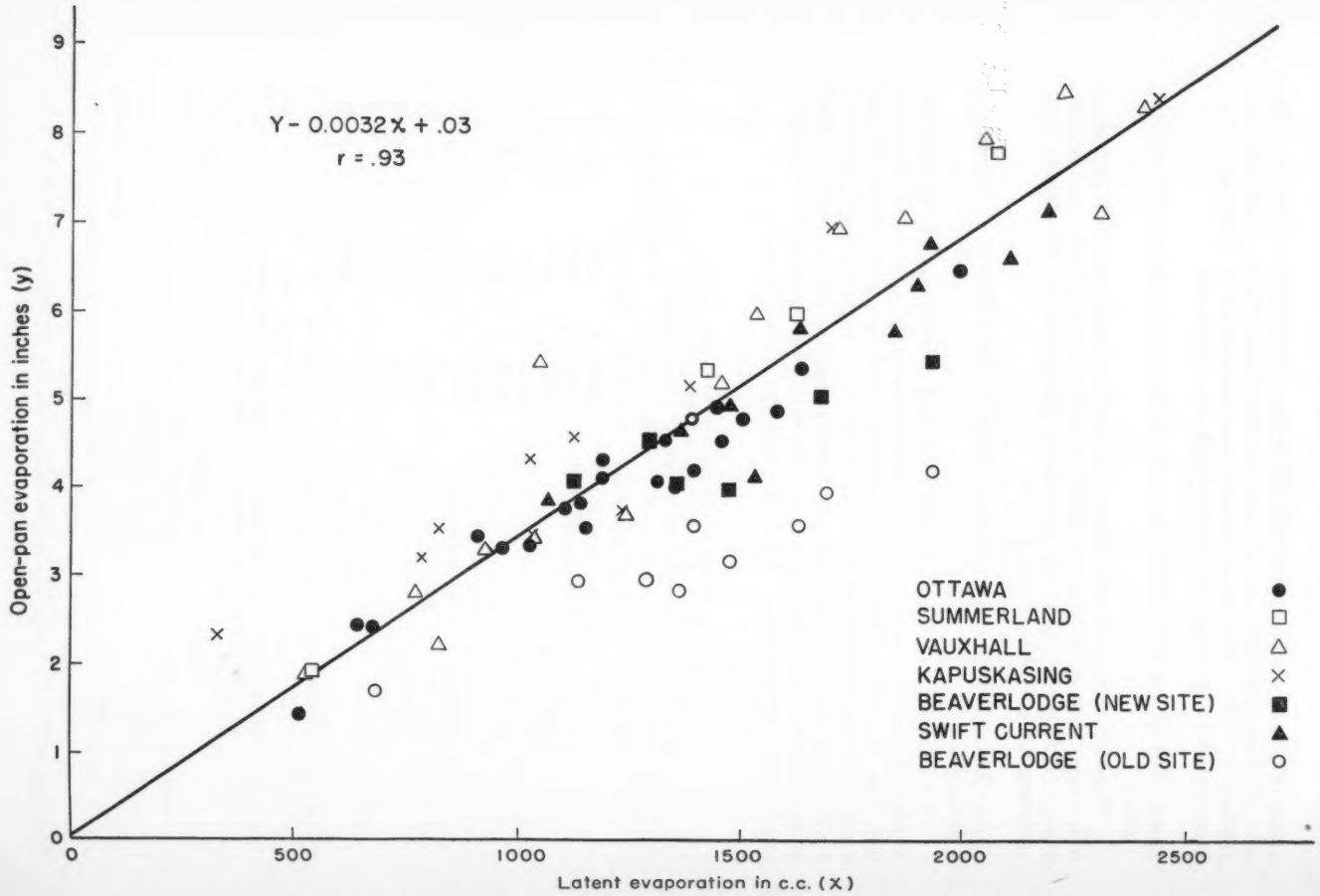

FIGURE 2. Scatter diagram of comparison between latent and open pan evaporation.

RESULTS AND DISCUSSION

Response of Various Evaporimeters to Daily Variations in Meteorological Factors

The rapid response of the black Bellani plate is indicated by the highly significant correlations with meteorological factors. The differences in construction of the various evaporimeters may partly account for the different correlation coefficients for each instrument. The Summerland tank is shielded from rain by a sheet of metal which obstructs the direct rays of the sun. The 4-foot tank is buried in the ground with the water level 2 to 4 inches below the rim which causes reduced air circulation. The colour of the white Bellani plate reduces the absorption of heat and the structure and exposure (within the Stevenson screen) of the Piché reduces its responsiveness.

The diurnal variation in latent evaporation (Figure 1) is typical of the daily transpiration cycle of plants, being lowest during the night, and highest in the afternoon. Latent evaporation continues during the night if drying conditions prevail (e.g. dry wind). Similarly, plant cuticular transpiration would continue during the night even though stomata are usually closed. Figure 1 indicates that the Bellani plate responds rapidly to changes in the drying ability of the air. It is generally known that pan-type evaporimeters are sluggish and tend to "carry-over" a great deal and may continue to evaporate at night only because of the heat absorbed during the day.

Comparison of Open-Pan and Latent Evaporation

The comparison of open-pan and latent evaporation is highly significant, and the slope of the line is 0.0032 (Figure 2). This amount was used for the conversion of latent to open-pan evaporation (in./c.c.). The y-intercept of .03 was found to be not significant. The open-pan at Beaverlodge on the "old" site was surrounded by trees and shrubs which reduced air circulation and hence evaporation. The ratio open-pan to latent evaporation was correspondingly reduced (Figure 2).

The variations in evaporation with geographic location can also be seen in Figure 2. The dry western stations have higher evaporation rates than the more humid eastern sites. There is a suggestion that ratios (open-pan/latent evaporation) at western stations may be higher than those at the more easterly sites. This may be partially accounted for by the so-called "oasis effect" occurring under dry conditions. At western stations, the pans are usually surrounded by dry soil and vegetation under high moisture stress. Consequently, with dry air passing over the ground, the lines of equal vapour pressure will have a sharp "hump" over the pan to a height of a few feet. The high vapour pressure gradients under those conditions would result in rapid evaporation. However, under humid conditions, the pan will have a negligible "hump" in the lines of equal vapour pressure above the surface because of the dampness of the surroundings. The lower gradient under these conditions results in less evaporation.

There is also a small "hump" in the lines of equal vapour pressure above the Bellani atmometer under both dry and humid conditions since the plate is supported 4 feet above the soil where the free air vapour pressure deficit during the daytime is usually greater than zero, regardless of surface

FIGURE 2. Scatter diagram of comparison between latent and open pan evaporation.

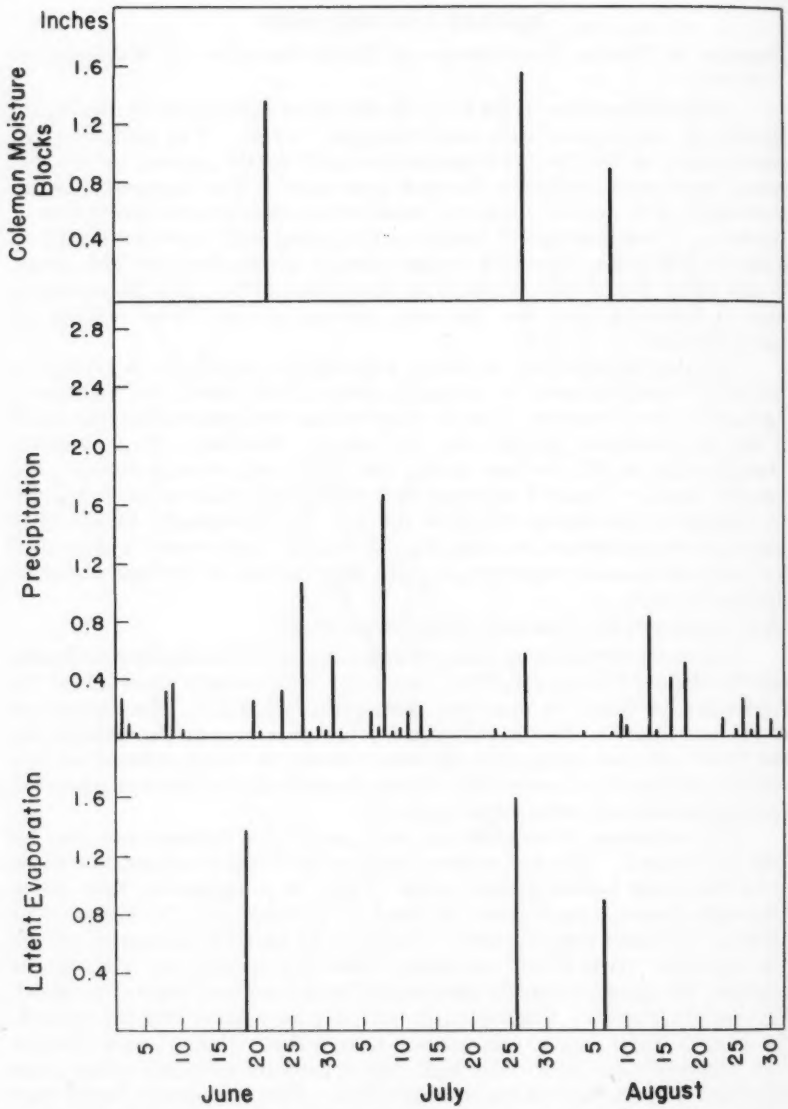

FIGURE 3. Amount of irrigation water required as indicated by the Coleman Moisture block and latent evaporation budget methods. (L'Assomption, 1956.)

moisture conditions. Therefore, there is less variation in the rate of evaporation from the Bellani plate than from the open-pan resulting from variations in the magnitude of the oasis effect. For this reason, evaporation from the Bellani atmometer supported 4 feet above the ground should

bear a more consistent relationship with potential evapotranspiration than does evaporation from open-pans buried in the ground. The over-all effect manifests itself in slightly higher ratios of open-pan to latent evaporation in arid than in humid areas.

Comparison of Latent Evaporation and Evaporation from Evapotranspirometers

At Swift Current the evapotranspirometers are surrounded by a narrow border crop. In addition the plants in the tank were not cut to the level of the border crop. This is reflected in the magnitude and variability of the P.E./L.E., while the ratio between open-pan and latent evaporation was lower and fairly constant throughout the summer (Table 3). The height of the crop above the border may be a determining factor as well as the actual border size. Air would pass through the crop as well as over and around it ("clothes-line effect"). Hence evapotranspiration was dependent on the height and age of the crop as well as on meteorological factors. At Kapuskasing, the border crop was large and was cut to the same height as the tank crop and the ratio P.E./L.E. was fairly constant throughout the season during both years.

The ratio of potential evapotranspiration to latent evaporation at Kapuskasing was found to be approximately 0.0035 (in./c.c.). This amount is somewhat higher than the value 0.0032 found for the ratio between open-pan and latent evaporation. The lower ratio of the latter may be due to lower pan evaporation because of rim turbulence of the pan, internal convection and radiative absorption by the containing walls of the pan.

Comparison of Latent Evaporation and Moisture Block Methods in Scheduling Irrigation

A conversion factor of 0.0034 inches of potential evapotranspiration for each cubic centimetre of latent evaporation was used in the Saanichton and L'Assomption irrigation experiments. This factor was based on the evapotranspiration data available at that time. Using this amount, the moisture block and latent evaporation methods at Saanichton (Table 5) required 9.0 inches of supplemental irrigation. The irrigation budget experiment at L'Assomption (Figure 3) was conducted during a wet season, and each method called for 4.0 inches of irrigation.

TABLE 5.—AMOUNT OF IRRIGATION REQUIRED BY THE COLEMAN MOISTURE BLOCK AND LATENT EVAPORATION BUDGET METHODS (SAANICHTON, 1956)

Coleman moisture block method		Latent evaporation method	
Date	Amount required (inches)	Date	Amount required* (inches)
May 7	field capacity		
May 19	1.0	May 22	1.0
June 4	0.4	May 31	1.0
July 3	0.5	July 3	1.0
July 9	1.5	July 13	1.0
July 23	1.0	July 20	1.0
July 31	1.3	July 28	1.0
Aug. 20	1.6	Aug. 20	1.0
Sept. 5	1.7	Sept. 3	1.0
		Sept. 5	1.0
	Total 9.0		Total 9.0

* Potential evapotranspiration was estimated by multiplying latent evaporation by .0034.

The difference between the ratios of evapotranspiration from evapotranspirometers and from irrigated field plots to latent evaporation may be significant. According to McLeod, potential evapotranspiration measured with evapotranspirometers is likely to be high (4). It is suggested, therefore, that 0.0034 is a more realistic ratio.

SUMMARY

Experiments with the black Bellani plate atmometer have shown it to be very responsive to meteorological factors, similar to plants in response to meteorological factors, reliable, easily installed and operated.

Latent and open-pan evaporation at Experimental Farms across Canada have shown a highly significant correlation. The ratio of 0.0032 inches of open-pan evaporation and 0.0034 inches of evapotranspiration from irrigated fields for each cubic centimetre of latent evaporation has been tentatively established.

A comparison of latent evaporation and moisture block methods in scheduling irrigation has shown excellent agreement, each method calling for comparable amounts of supplemental water.

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CONTROL OF STEM AND LEAF RUST OF WHEAT WITH FUNGICIDES[1]

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ABSTRACT

The results of field evaluations of fungicides for the control of stem and leaf rust of wheat at Winnipeg are presented. Economical control was achieved by means of chemicals, especially nabam plus zinc sulphate, provided that the rainfall was not adverse during the period of the spray program. Zineb was as effective as nabam plus zinc sulphate but was considerably more expensive. Four to five applications each of 1½ quarts (Imp.) nabam (19 per cent) and ¾ pound zinc sulphate per acre gave effective control. It was extremely important to begin application of the chemicals early, when only a trace of rust was present.

The problem of controlling the cereal rusts with protective fungicides is discussed.

INTRODUCTION

Inorganic chemicals, of which sulphur dust was the most effective, were used in the early efforts to control rust by chemical means. The work of Kightlinger (5), Lambert and Stakman (6), Bailey (2) and Greaney (3) showed that if sulphur dust were kept on the leaf surface by repeated applications of 30 to 40 lb. per acre, yield increases of from 3 to 30 bushels per acre could be realized.

When stem rust-resistant varieties of wheat became generally available, in 1939, interest in rust control by chemical means diminished and few rust control chemicals were tested during the next decade. However, with the advent of race 15B of stem rust of wheat (*Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn.) interest was revived in the control of cereal rusts with fungicides.

Recently there has been a trend towards the investigation of organic chemicals as fungicides. Some of these fungicides act by protecting the host from infection and others act by therapy, the effect occurring inside the host. The terminology of Horsfall (4) regarding the meaning of plant protection and therapy is followed in this paper.

The majority of the chemicals which have been used to date in rust control have been of the protective type, acting only on the surface of the plant. The sulphur dusts and sprays, the carbamates (e.g. nabam, ferbam, maneb and zineb), the naphthoquinones (e.g. dichlone) and the crotonates (e.g. Karathane) are of this type.

Several of the fungicides which act by therapy have been tested but only calcium sulfamate has been reported to be satisfactory for rust control (7). Unfortunately, calcium sulfamate has a harmful effect on the wheat plant and on the germination of seed harvested from treated plants (1).

[1] Contribution No. 1629 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ont.

[2] Plant Physiologist and Plant Pathologist, respectively, Plant Pathology Laboratory, Winnipeg, Man.

The antibiotic actidione may act systemically. Livingston (7) and Wallen (10) have reported promising results with actidione in the control of stem rust of wheat. However, actidione is phytotoxic if a slight overdose is applied.

Each year tests are made at Winnipeg in field plots to evaluate the effectiveness of the new fungicides in the control of stem rust and leaf rust of wheat. The results of these tests for the years 1952-56 inclusive are presented.

MATERIALS AND METHODS

The chemicals,* which have been evaluated at Winnipeg, have all been supplied by commercial manufacturers of fungicides after tests by the companies had demonstrated the effectiveness of the fungicides in rust control. A description of the materials used is given in Table 1.

Greenhouse tests, designed to give useful information about rates of application of a chemical for field trials, were made when a chemical was received for the first time. Seedlings of wheat were grown to the first leaf stage. The protective fungicides were sprayed on the seedlings with a hand atomizer until the solution began to run off the leaves. Approximately half the solution was applied from one direction and half from the opposite direction. The leaves were then allowed to dry. Urediospores of stem rust or leaf rust, mixed with talcum powder (1 in 20 by volume), were dusted on the leaves and the plants were incubated in a moist chamber for 24 hours. When the uredial infections had broken the leaf epidermis a count was made of the total number of infections occurring per leaf. The relative effectiveness of the chemical as a protective type of fungicide was recorded.

If a chemical was to be tested as a therapeutic type of fungicide, the plants were inoculated and incubated as above and the chemical was applied by atomizer to the leaves after the infections became apparent (one or two days after flecking). The chemical was considered to have therapeutic properties if the development of the rust pustules was arrested.

In the field plot experiments, the wheat variety Red Bobs was used in 1952, Marquis in 1954 and 1955, and Thatcher in 1956. The wheat was grown in rows 1 foot apart, in plots 4 feet by 18 feet. Usually four buffer rows were left between each pair of plots. There were four replicates of plots for each check and for each fungicidal treatment. The locations of the plots within a block were chosen at random with each block containing one plot of each treatment and one control plot.

Infection by stem rust and leaf rust was due entirely to naturally occurring inoculum in the years 1952 to 1955. A leaf rust epiphytotic was artificially induced in the field plots in 1956.

The fungicidal dusts were applied with a Niagara Cyclo-Junior duster in the early morning while the plants were moist with dew. The sprays were applied with 1- or 2-gallon capacity knapsack sprayers of various makes, none of which had a gauge to indicate the pressure within the tank.

* The coined and trade names for the organic fungicides used in this paper refer to the following compounds (sources in parentheses): C.M.U.—p-chlorophenyl dimethylurea, dichlone-2,3-dichloro-1,4 napthoquinone obtained as Phygon, maneb-manganese ethylene bisdithiocarbamate, nabam-disodium ethylene bisdithiocarbamate, calcium sulfamate and zineb-zinc ethylene bisdithiocarbamate (obtained from E I. du Pont de Nemours and Co.); Karathane—dinitro (1-methylheptyl) phenyl crotonate and nabam (obtained from Rohm and Haas Co.); Sulforon—sulphur (Niagara Brand Spray Co.); Thioneb-ethylene thiuram monosulphide polymerized (Naugatuck Chemicals); sodium sulfanilate (Monsanto Chemical Co.)

The size of the water droplets produced by the sprayers was rather large, with the result that effective coverage of the plant surfaces was not obtained with less than 66 gal. (Imp.) of solution per acre.

Certain computations and assumptions were necessary in order that the amounts of fungicide and of water which were applied to small plots could be related to large-scale practice. The area of one plot, consisting of four 18-foot rows, was 72 square feet (1/605 of an acre). Whenever specifications required 1 lb. (453.6 gm.) per acre of chemical the amount actually used was 0.75 gm. per 4 rod-row plot. For 100 gal. (Imp.) of solution per acre, 751.4 ml. per 4-row plot was used. A rate of application of 500 ml. per 4 rod-row plot was considered to be equivalent to 66 gal. per acre.

The total number of applications of a fungicide in a given season depended on the type of control expected. For instance, zineb dust and spray, Sulforon dust and spray, maneb dust and spray, dichlone dust and spray, Karathane powder and emulsion, Thioneb and nabam were all considered to be protective in effect and were applied at 7- to 9-day intervals after the spray program was started.

Calcium sulfamate had been tested in the greenhouse and found to be capable of inactivating stem rust in wheat leaves at any time between 24 hours after inoculation and the time of the breaking of the epidermis by the rust infection. There was considerable dessication of the infection, even after the epidermis of the leaf was broken. This chemical was used in the field with longer intervals between applications than were used with the protective type of fungicides.

The CMU was considered to be systemic in action and was applied to the soil by pouring the solution on the soil between the rows when the seedlings were in the 5-leaf stage.

Several criteria were used to evaluate the effectiveness of the chemicals under test. They were: the percentage of rust at the final reading by use of the modified Cobb's scale (9); the 1000-kernel weight; the yield in bushels per acre; the value of the increase in yield (if any), and the estimated profit per acre based on a price of $2.00 per bushel.

In 1955 and 1956 the fungicide nabam, a 19 per cent liquid formulation of the disodium salt of ethylene bisdithiocarbamate, was used with zinc sulphate. This combination was cheaper and easier to apply than were the wettable powders which tended to clog the spray nozzles, a difficulty not experienced when nabam plus zinc sulphate were used.

In 1955 and 1956 the total number of applications of the protective type of fungicide was varied to determine at what stage of wheat maturity and of rust infection the fungicide application should begin and how many applications would be most profitable.

A test of the germination of the harvested grain from many of the treatments was made.

RESULTS

The results of the field plot evaluations of fungicides at Winnipeg from 1952 to 1956 inclusive, excepting 1953, are presented in Table 1.

The effectiveness of the protective type of fungicide varied from year to year. In 1953 the use of these fungicides did not increase the yield

TABLE 1.—RESULT OF APPLICATIONS OF FUNGICIDES FOR RUST CONTROL ON FIELD PLOTS OF WHEAT[x] AT WINNIPEG 1952-56[xx]

Treatment	lb./acre	Number of applications	Av. 1000-kernel wt. of check and diff. from check	Yield of check in bu./acre and diff. from check	Value of increase at $2.00/bu.	Cost of chemical per acre	Profit per acre†	Percentage rust, final reading
		1	2	3	4 $	5 $	6 $	7
1952								
Check			14.7	15.4				heavy
Zineb dust	40	5	+18.6**	+21.5**	43.00	32.00	11.00	very light
Zineb spray	1½	5	+16.3**	+20.3**	40.60	10.00	30.60	very light
Sulforon dust	40	5	+14.2**	+16.7**	33.40	14.00	19.40	very light
Maneb dust	40	5	+ 9.2**	+13.2*	26.40	32.00	− 5.60	light
Maneb spray	1½	5	+ 7.6**	+11.7	23.40	10.00	13.40	light
Phygon dust (dichlone)	40	5	+ 8.4**	+10.8	21.60			mod. heavy
Phygon spray (dichlone)	1½	5	+ 3.3*	+ 5.5	11.00	12.90	− 1.90	mod. heavy
Calcium sulfamate dust	40	5	+ 9.7	− 0.2				light
1954								
Check			24.6	22.1				59
C.M.U.	1	1	− 1.4	+ 1.3	2.60			59
C.M.U.	2	1	+ 0.5	+ 1.4	2.80			62
Calcium sulfamate	8	2	− 0.9	− 1.9	− 3.80	5.60		30
Karathane	3	2	+ 1.4	+ 5.5*	11.00	12.80	− 1.80	50
Karathane	3	3	+ 4.3*	+ 4.1*	8.20	14.40	− 6.20	52
Karathane	2	3	+ 3.8*	+ 7.4**	15.80	9.60	+6.20	47
Ca. sulfamate (8)[1] & Na. sulfanilate (6)[2]		1	+ 2.4	+ 3.9	7.80			53
Ca. sulfamate (8)[1] & Na. sulfanilate (6)[2]		2	+ 1.1	+ 2.5	5.00			37
Sodium sulfanilate	6	2	− 0.2	+ 1.9	3.80			50
Ca. sulfamate (8)[1] & then Na. sulfanilate (6)[2]		1 ea.	− 0.2	− 0.1	− 0.20			39
Na. sulfanilate (6)[2] & then Ca. sulfamate (8)[1]		1 ea.	+ 0.4	+ 0.9	1.80			57

1955								
Check			24.7	17.8				65
Thioneb (50%)	1½	8	+ 4.6**	+10.4**	20.80	8.00	12.80	15
Thioneb (50%)	1½	7	+ 4.8**	+ 9.9**	19.80	7.00	12.80	25
Thioneb (50%)	1½	5	+ 4.6**	+ 7.4**	14.80	5.00	9.80	40
Thioneb (50%)	1½	4	+ 3.0**	+ 5.0*	10.00	4.00	6.00	45
Thioneb (50%)	1½	3	+ 1.1	+ 2.5	5.00	3.00	2.00	65
Nabam plus ZnSO4	2.24††	5	+ 4.3**	+ 5.2**	10.40	10.00	0.40	10
Nabam plus ZnSO4	1.12	5	+ 2.6*	+ 2.8*	5.60	5.00	0.60	25
Nabam plus ZnSO4	0.56	5	+ 1.0	+ 1.9	3.80	2.50	1.30	35
1956								
Check			30.1	43.9				80
Nabam plus ZnSO4 July 6, 13, 23, 31, Aug. 9	1.2††	5	+ 4.4**	+10.6**	21.26	5.00	16.26	5
Nabam plus ZnSO4 July 6, 13, 23, 31	1.2	4	+ 4.3**	+10.8**	21.62	4.00	17.62	7
Nabam plus ZnSO4 July 6, 13, 23	1.2	3	+ 4.0**	+ 8.2**	16.42	3.00	13.42	11
Nabam plus ZnSO4 July 6, 13	1.2	2	+ 2.1**	+ 7.5**	14.96	2.00	12.96	57
Nabam plus ZnSO4 July 6	1.2	1	+ 1.1	+ 2.5	5.02	1.00	4.02	60
Nabam plus ZnSO4 July 13, 23, 31, Aug. 9	1.2	4	+ 4.6**	+ 6.1	12.28	4.00	8.28	11
Nabam plus ZnSO4 July 23, 31, Aug. 9	1.2	3	+ 2.7**	+ 0.1	0.28	3.00	− 2.72	42
Nabam plus ZnSO4 Aug. 9	1.2	1	+ 0.3	+ 0.4	0.74	1.00	− 0.26	75

x Variety Red Bobs 1952, Marquis 1954, 1955 and Thatcher 1956.
xx There were not any results in 1953.
* Significant at 5% level
** Significant at 1% level
† Profit before deducting cost of application.
†† Pounds of resulting Zineb.
1 8 lb./acre.
2 6 lb./acre.

The chemicals used and the percentage of active ingredient were: zineb (zinc ethylene bisdithiocarbamate) dust 5.2%, zineb for spraying 65% wettable powder, sulforon dust 100%, maneb (manganese ethylene bisdithiocarbamate) dust 5.2%, maneb for spraying 65% wettable powder, dichlone (2,3-dichloro-1,4 naphthoquinone) dust 4.0%, dichlone for spraying 37% wettable powder, calcium sulfamate dust 15%, C.M.U. (3- (p-chlorophenyl) -1, 1-dimethylurea) 80% wettable powder, calcium sulfamate for spraying 100%, Karathane (dinitro (1-methylheptyl) phenyl crotonate) 22.5% wettable powder, sodium sulfanilate 100%, Thioneb (ethylene thiuram monosulfide polymerized) 50% wettable powder, nabam (disodium ethylene bisdithiocarbamate) 19% and zinc sulphate 36% metallic zinc.

because heavy and frequent rains removed the fungicides from the surfaces of the plants shortly after each application. The dust form of the protective fungicides was more expensive to use than the sprays because of the large volume of inert material which had to be shipped and handled.

Zineb (65 per cent) wettable powder has been found to be a very successful protective fungicide against stem rust and leaf rust of wheat in greenhouse and field tests. Therefore, whenever a newly developed protective fungicide was to be tested in the greenhouse it was compared with zineb. Thioneb (50 per cent) was tested in the greenhouse by using the same weight of active ingredient as of zineb and a series of dilutions of each was used. Zineb (65 per cent) wettable powder, was used at the rate of 0.168 gm. per 95 ml. and Thioneb (50 per cent) at 0.218 gm. per 95 ml. with one drop of Triton B-1956 spreader-sticker. The greenhouse tests indicated that the Thioneb was at least four times as effective as the zineb, weight for weight of active ingredient. Nevertheless, Thioneb was not so effective in field tests as would have been expected on the basis of the greenhouse trials (see Table 1). The reason for its reduced activity in the field was not ascertained.

The zineb wettable powder is insoluble and must be prepared in the form of a slurry to avoid clogging of the spray nozzles. This fact, combined with the lower cost of nabam, led to the use of nabam plus zinc sulphate as the main protective type of fungicide in the 1956 field trials. Four to five applications of 1½ Imp. qt. nabam (19 per cent) plus 3/4 lb. zinc sulphate per acre per application gave effective control. Although 66 gal. of water per acre were used, because of the coarse spray from the knapsack sprayers, the use of 20 to 40 gal. of water per acre might be sufficient if applied as a fine spray. One or two ounces of a spreader-sticker such as Triton B-1956 is recommended for effective coverage.

The test of germination of seed (see Table 2) from treated plants revealed that of the fungicides used only calcium sulfamate reduced germination.

TABLE 2.—EFFECT OF FUNGICIDES APPLIED FOR RUST CONTROL ON THE GERMINATION OF SUBSEQUENTLY HARVESTED GRAIN*

Chemical used	Amount applied per acre	Number of applications	Percentage germination of seed		
			1953	1954	1955
Check			76	72	86
Zineb 65% wettable powder	1½ lb./100 gal.	5	72	—	—
Karathane (22.5%) wettable powder	2 lb./100 gal.	3	—	77	—
Karathane (22.5%) wettable powder	3 lb./100 gal.	2	—	81	—
Sodium sulfanilate later Calcium sulfamate	7 lb. 15 lb./10 gal.	2	—	24	—
Calcium sulfamate	8 lb./10 gal.	2	—	30	—
Calcium sulfamate	15 lb./10 gal.	1	5	—	—
Calcium sulfamate dust	40 lb.	5	39	—	—
Calcium sulfamate and Sodium sulfanilate	8 lb. 6 lb./10 gal.	1	—	64	—
Calcium sulfamate and Sodium sulfanilate	8 lb. 6 lb./10 gal.	2	—	14	—
Sodium sulfanilate	6 lb./10 gal.	2	—	80	—
Thioneb (50%)	1½ lb./66 gal.	8	—	—	92
Thioneb (50%)	1½ lb./66 gal.	5	—	—	91

*Wheat variety Red Bobs 1953, Marquis 1954, 1955

DISCUSSION AND CONCLUSIONS

The results of the field plot experiments indicate that economical control of stem rust and leaf rust of wheat can be obtained with the best protective type of fungicide, provided that weather conditions do not become very adverse during the spray program. In 1953 none of the chemicals used (including zineb and sulphur) was successful in controlling rust because of frequent heavy showers during the period of fungicide application. This failure emphasized one of the basic difficulties in the use of the protective type of fungicide, which is the need to re-apply the chemical to the plant surfaces after a heavy shower of rain. A very efficient spreader-sticker might partially overcome this difficulty.

Calcium sulfamate controls rust by inactivating the fungus within the host tissue. However, the sulfamate reduces the milling quality of the grain and it also lowers the percentage of germination (8) and (1). A chemical as effective in therapy as calcium sulfamate and with more protective action would go a long way towards filling the requirements of the ideal fungicide for cereal rust.

The test in 1955 with Thioneb showed that the yield and the profit per acre (if the cost of labour is not included) both increased with increasing number of applications up to a total of seven. However, as there were no plots in this test which received early sprays only, no information was obtained on the effectiveness of early versus late sprayings.

In 1956, some plots received both early and late sprays, others early sprays only, and still others late sprays only. The results in 1956 illustrated that the most economical treatment was the one in which the first four applications of fungicide were made early; the first two applications seemed to be the most important in preventing reduction in yield. These experiments made it clear that it is extremely important to begin application of the chemicals early, when only a trace of rust is present. When late sprays only were used the profit per acre was greatly reduced.

In search for a fungicide highly satisfactory for rust control it should be borne in mind that the cost of the chemical and its application must be reasonable. The cost of sulphur dust for five applications of 40 lb. per acre is approximately $14.00 per acre. The cost of nabam (1½ Imp. qt.) plus zinc sulphate (¾ lb.) for five applications per acre is approximately $5.00 per acre. One of the objectives in testing new protective fungicides for rust control is to reduce the cost of protection.The cost of applying the chemical is difficult to assess since the type of sprayer in use and the value of the operator's labour are variables. An estimate of 25 cents per acre to apply 2,4-D with ground equipment has been reported. The sprayers which are drawn by tractors are not the best possible equipment for applying chemicals to grain in the heading stage. However, a new self-propelled sprayer with a high chassis is in limited use in Western Canada and would seem to be suitable for the purpose.

The use of aeroplanes to apply fungicides would be very costly if more than 5 gal. of liquid were necessary per acre. Costs as low as $1.00 per acre per application are usual when weed-killers are applied by air at the rate of 2 or less gallons of solution per acre[1].

[1] Cited by Wood, H. E., Secretary, Manitoba Weeds Commission, Publications Branch, Man. Dept. Agr., Winnipeg, Man. *Private communication*, June 1956.

Wheat growers object to the damage caused by the wheels of the spray equipment. A type of strip farming with 50- to 60-foot strips of legumes sown with the wheat, so that the wheat strip becomes the legume strip in crop rotation, might be best. This would give firm support for the wheels, a paying legume crop, put nitrogen into the soil, and eliminate wheel damage to the wheat. The boom of the sprayer would extend 25 to 30 ft. over the wheat strip as the sprayer is driven along on the legume strip.

Desirable properties of a protective fungicide for rust control are:

1. Low cost and/or a high level of effectiveness in low concentration
2. Long storage without deterioration
3. Lack of phytotoxicity
4. Little or no residue in the soil if the chemical is phytotoxic to wheat or other plants of commercial value
5. No toxic residue on the seed
6. No undesirable effect on the baking quality of the flour
7. No undesirable effect on germination of the seed harvested from treated plants.

The work here reported has made it clear that a susceptible variety of wheat can be protected against stem rust and leaf rust economically with protective fungicides. If, however, resistant varieties are available, it would seem more practical at the present time to grow them than to protect susceptible varieties by chemical means.

This paper has dealt mainly with the results of attempts to control cereal rust with protective fungicides. A report of tests with recently developed fungicides which act by therapy will be published at a later date.

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A TEST SILO AND APPARATUS FOR ENSILING STUDIES[1]

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ABSTRACT

Special equipment was developed to facilitate the ensiling and study of ensiled crops in small lots. A description is given of the design and use of an experimental metal silo, as well as the apparatus required for compacting the silage, weighing and moving the silo. Plastic sheeting greatly reduced the average silage losses while sawdust reduced somewhat the loss.

INTRODUCTION

Experiments dealing with the ensiling of crops have been conducted in a variety of silo types, with storage capacities ranging from a few pounds to many tons of crop. Perkins (5) and Kennedy (4) have reported silage experiments using relatively small laboratory silos. Barnett (1) and Watson (6) have published results obtained with silos of half-ton to three tons capacity. The ensiling equipment herein described is designed for storage of approximately one-half ton green weight of crop. Such a silo has most of the advantages of a laboratory silo and closely simulates conditions that occur in farm-scale silos.

MATERIALS AND METHODS

A metal silo, 48 inches high and 37 inches in diameter, was constructed (Figure 1). The sidewalls were built of 1/8-inch sheet iron, while 3/16-inch material was used for the bottom. The top of the silo wall was reinforced with an iron band and all joints were electrically welded. Lifting lugs were installed near the top of the silo wall. Threaded plugs were located in the central area of the sidewalls for sampling during the ensiling period. Free silage juices were collected by means of a drainage trap placed at the bottom of the sidewall. The silo was treated with acid-resistant paint prior to its general use. A combination hoist and press is mounted on a metal frame to facilitate the actual ensiling operations. Four castor-type wheels allow the frame to be hand-moved in any direction. The compacting device consists of hand-operated hydraulic pumps and two interconnected rams, which act on a reinforced pressure place fitting inside the silo. This unit can exert a pressure of 37 lb. per square inch of plate area. Provision is thus made for a method of compacting ensiled crops to an initial density of 40 to 50 lb. per cubic foot, depending on the particular ensiling conditions. The lifting and lowering of a silo is accomplished by means of a mechanical jack connected to a horizontal yoke equipped with lifting links. By lowering the yoke, the links can be hooked to the silo lugs and the silo lifted by means of the jack.

In the ensiling operations, the silo is placed on a wooden stand adjacent to a wagon-load of forage. The forage is forked into the silo and tramped until the silo is filled. The portable frame is then moved into position

[1] Contribution from the Field Husbandry, Soils and Agricultural Engineering Division, Experimental Farms Service.

and the silo lowered on to supporting cross members of the frame. The pressure plate is placed on top of the silo and the material compressed to the limit of the ram by the hydraulic pump. After releasing the pressure, the plate is removed and the operations repeated. Refilling is continued until the required amount is compressed into the known silo volume to obtain the desired initial density. By lowering the silo to a platform scale moved beneath the portable frame, the weight of the ensiled crop is readily checked. Plastic-covered thermocouple wires are used to measure the silage temperature. These are installed while the silo is being filled and may be placed at any desired location. To obtain temperature readings, the thermocouple leads are connected to a direct reading potentiometer. Where many readings are desired, considerable time can be saved by the use of multiple point switches.

Following the filling operations the silo is covered and then moved to wooden stands for storage. With the aid of the portable frame, losses in weight during ensiling may be checked by weighing on platform scales.

RESULTS AND DISCUSSION

In 1953 and 1954 the experimental tanks were used to study the effects of various types of silo covers on fermentation and spoilage losses. Results of these trials are shown in Table 1.

TABLE 1.—THE USE OF VARIOUS COVERING MATERIALS AS RELATED TO ENSILING LOSSES OF FORAGE CROPS*

Silo covering	Total forage ensiled	Total resulting silage	Edible silage	Spoiled silage	Silage losses—percentage of total material ensiled		
					Spoilage	Fermentation	Total
	lb. D.M.	lb. D.M.	lb. D.M.	lb. D.M.	%	%	%
			1953				
No cover	308.9	257.5	205.1	52.4	17.0	16.6	33.6
Sawdust	363.3	296.5	263.0	33.5	9.2	18.4	27.6
Plastic	235.5	220.2	208.9	11.3	4.8	6.5	11.3
			1954				
No cover	200.6	163.8	128.7	35.1	12.5	18.3	30.8
Sawdust	222.0	193.2	164.3	28.9	13.1	13.0	26.1
Plastic	224.7	217.0	201.8	15.2	6.7	3.4	10.1

* Single determinations

The above data show that forages ensiled without covers had an average loss of about 32 per cent. A covering of plastic sheeting reduced this loss to about 11 per cent. Forages ensiled and covered with wetted sawdust had an average loss of about 27 per cent.

Some results of initial experiments conducted with these experimental silage tanks have been reported previously (2) and (3).

The development of this equipment provides a useful technique for studying various aspects of silage preservation. With such a silo, forages

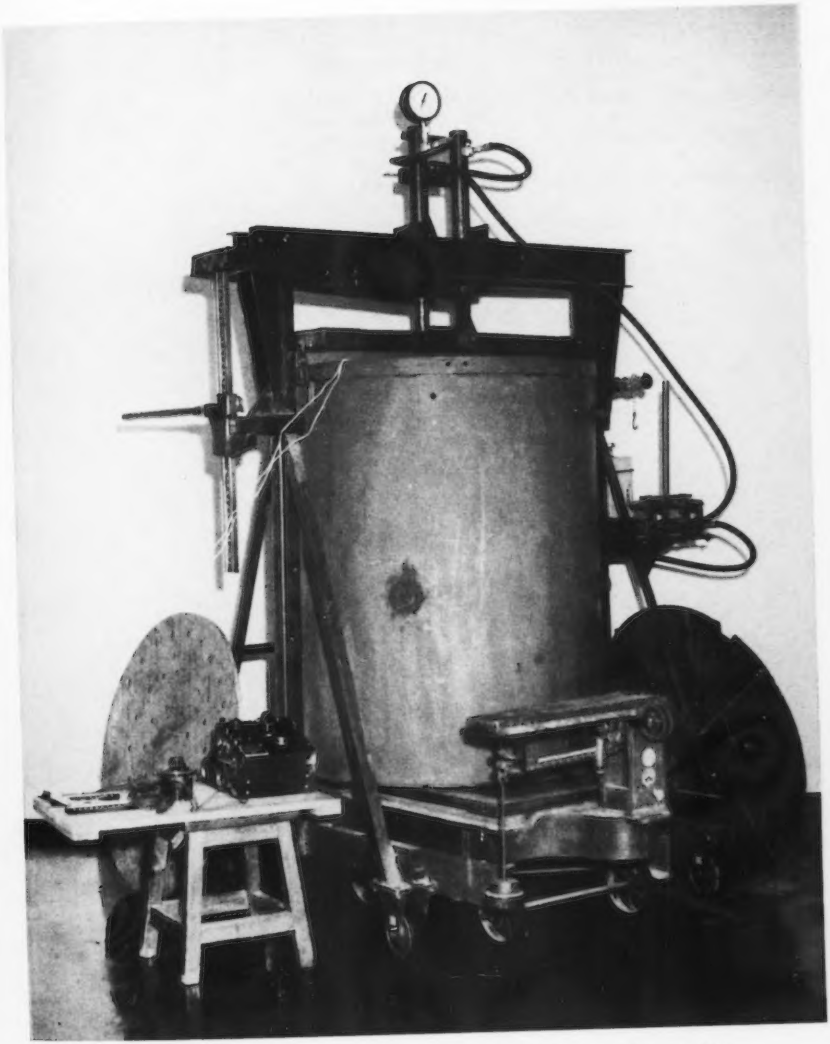

FIGURE 1. Metal tank and apparatus used in silage experimentation.

undergo normal ensiling in relatively small amounts, allowing for replicated treatments of uniform material. By means of the compacting device, a wide range of silage densities is obtained and pressures that normally occur in a farm-scale silo are simulated. The portable frame and hoist eliminate considerable labour and generally accelerate the ensiling operations, especially where a number of such silos are to be filled.

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A MONOSOMIC ANALYSIS OF STEM RUST REACTION AND AWN EXPRESSION IN REDMAN WHEAT[1]

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ABSTRACT

Using the recently developed monosomic series of Redman spring wheat in crosses with Prelude, genetic studies were undertaken on stem rust reaction and awn expression. It was found that chromosomes III, VIII and XIII of Redman carry factors for adult plant resistance to race 56 of *Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn. These factors are complementary and dominant in action. Complementary dominant factors for awn suppression appear to be located on chromosomes IX and XVII. These results are discussed in the light of recent comparable monosomic studies.

INTRODUCTION

Campbell (2) tentatively concluded that two complementary factors for adult plant resistance to stem rust are involved in the cross between the Chinese Spring and Redman varieties of *Triticum vulgare* Vill. and that chromosomes III and V carry these factors. This study involved the use of the monosomic lines of Chinese Spring and the methods described by Sears (7). No other genetic study of stem rust reaction in Redman has been published.

Numerous genetic studies have been made on the stem rust resistance of H-44-24, the resistant parent of Redman. The conclusions of these studies varied depending on the cross involved, but in general indicated that H-44-24 has either two independently inherited dominant factors each giving adult plant stem rust resistance or a single dominant factor (1). Recently Sears *et al.* (8) found evidence that the variety Hope, a sister selection of H-44-24, has factors conditioning adult plant resistance on chromosomes III and XVII, while VIII and XVII carry genes for seedling resistance. In addition, studies (8) conducted on Thatcher, which has Marquis and Kanred in its parentage in common with Redman, indicated that factors on chromosomes III and XIII may be responsible for adult plant resistance to certain races of stem rust.

Watkins and Ellerton (11) concluded that allelic series at three loci accounted for the different awn types in hexaploid wheats. A number of studies involving the Chinese Spring monosomics have verified these results. Major factors affecting awnedness have been located on chromosomes VIII, IX and X (3, 4, 6, 10). Minor factors conditioning awn expression have been found on chromosomes II and XX (3, 6, 12).

MATERIALS AND METHODS

Redman spring wheat is a derivative of the cross Reagent × Canus, that is, (H-44-24 × Reward) × (Marquis × Kanred). In 1951 a program was initiated to develop the 21 possible monosomic lines in Redman using Sears' Chinese Spring monosomics (6). By 1955, all 21 lines had been backcrossed

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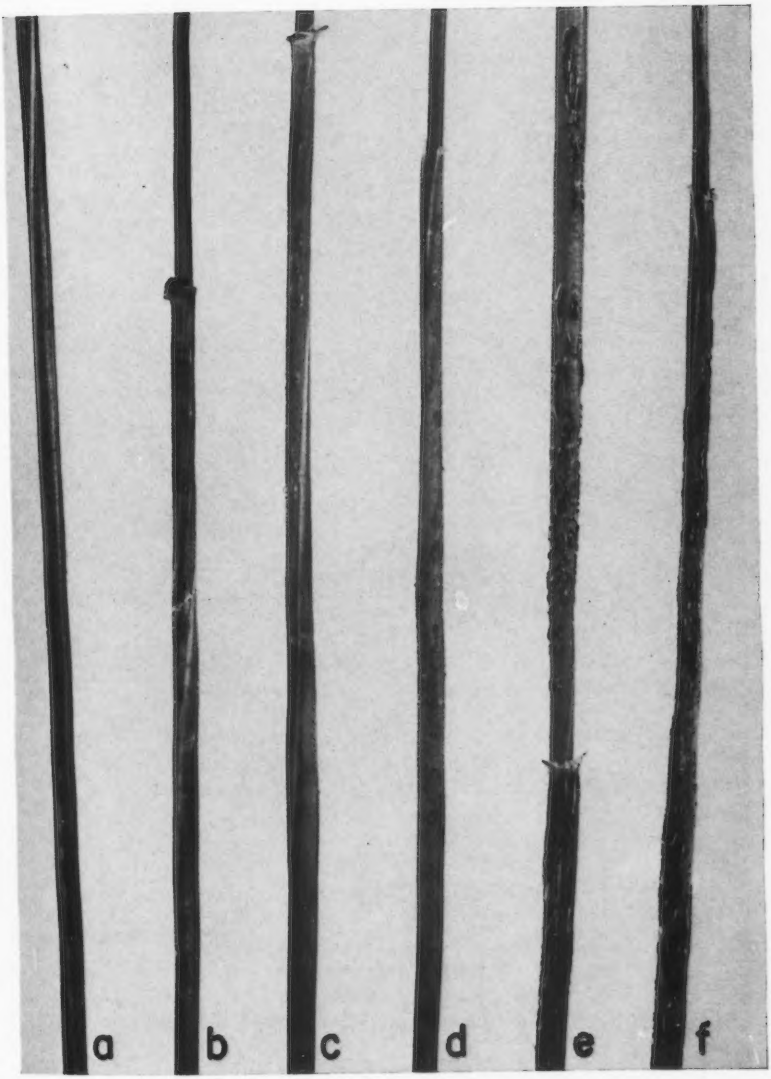

FIGURE 1. Typical rust reactions of parents, non-critical and critical lines.
a. Redman.
b and *c*. Normal and monosomic, respectively, in a non-critical line.
d and *e*. Normal and monosomic, respectively, in a critical line.
f. Prelude.

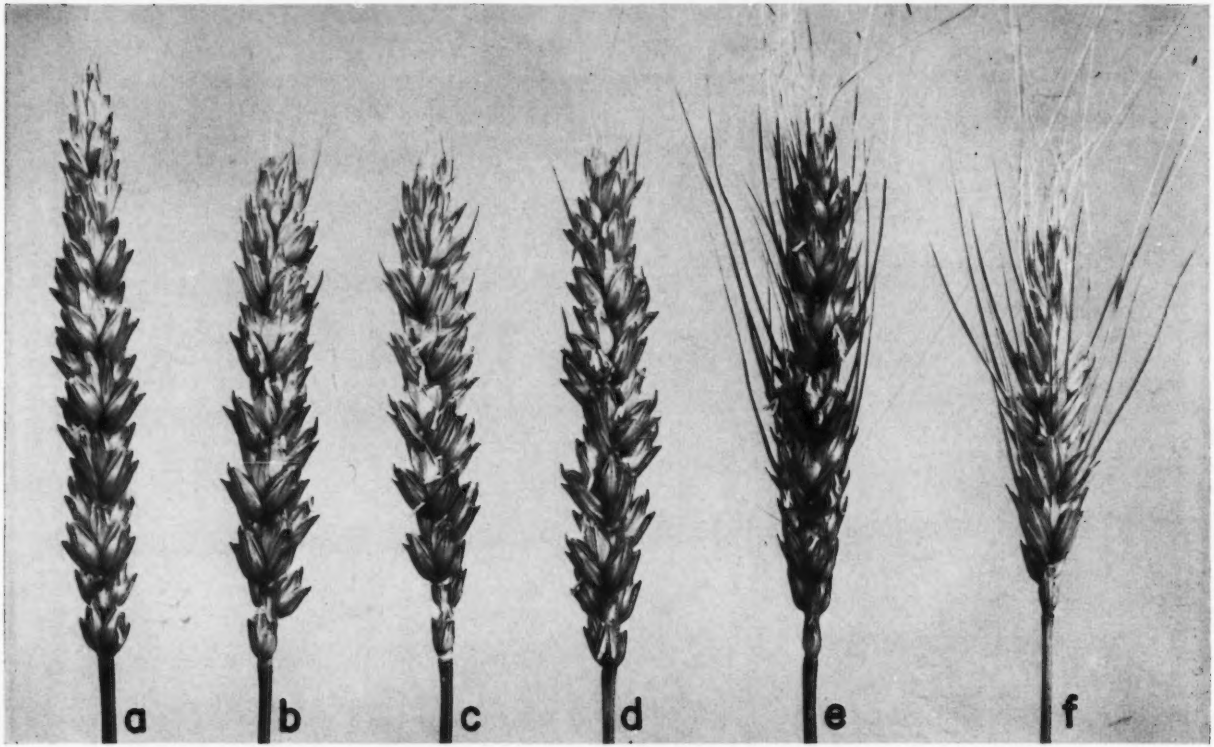

FIGURE 2. Typical awn expression in parents, non-critical and critical lines.

a. Redman.
b and *c*. Normal and monosomic, respectively, in a non-critical line.
d and *e*. Normal and monosomic, respectively, in a critical line.
f. Prelude.

to Redman five times and were morphologically similar to it. During the backcrossing program the monosomics were identified cytologically. These 21 Redman monosomics were crossed with Prelude, a fully awned variety that is susceptible to most races of stem rust. Where possible, 6 or more F_1 plants of each line were grown. In a few lines, however, only 4 or 5 plants were available. All F_1 plants were inoculated in a greenhouse or growth cabinet with a culture of race 56 of *Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn. and were subsequently scored on pustule type (9). In addition, the chromosome number of each plant was determined cytologically.

Chromosomes of Redman carrying factors for resistance to race 56 were identified in the following manner: If resistance to race 56 in Redman is conditioned by one dominant factor or two or more complementary dominant factors, the absence of any one factor would be manifest by a susceptible reaction in the critical monosomic F_1 plants. Normal plants from the critical line would be resistant, since each would possess the Redman factor or factors for resistance. If, on the other hand, resistance is recessive or conditioned by duplicate dominant factors, disturbed ratios would occur in the F_2 populations of the lines involving the critical chromosomes.

The same F_1 populations of the crosses between Redman monosomics I to XXI and Prelude described above were used to determine which chromosomes carry factors for awn suppression in Redman. All plants were classified according to parental awn type; that is, whether fully awned like Prelude or awnletted like Redman. Identification of the chromosomes involved was made on the same basis as that used for rust reaction.

RESULTS

Prelude exhibited a susceptible adult plant reaction (type 4 pustule) to race 56 of stem rust, while Redman gave a resistant reaction (type ; and 1). Monosomic F_1 plants which were deficient for chromosomes III, VIII or XIII of Redman were as susceptible as Prelude, while normal plants from these three lines and all other F_1 plants were resistant, with the exception of one plant in each of lines I and XX (Table 1). The differences in rust reaction are shown in Figure 1.

All of the F_1 monosomic plants in lines IX and XVII and 4 of the 12 monosomics in line XXI were fully awned. The normal plants from crosses involving these 3 chromosomes and all other F_1 plants, including the remaining 8 plants deficient for chromosome XXI, were awnletted. Two of the awned plants deficient for XXI were studied further and were found to be heterozygous for a reciprocal translocation. The other 2 awned plants, unfortunately, could not be re-examined. The differences in awn expression are shown in Figure 2.

DISCUSSION AND CONCLUSIONS

The fact that monosomic F_1 plants from crosses involving chromosomes III, VIII and XIII were susceptible and that disomic plants were resistant, indicates that these chromosomes in Redman carry dominant factors for resistance to race 56. Furthermore, these factors must act in a complementary manner, since the absence of any one completely inhibited the

expression of resistance of the others. All 3 of these chromosomes have been associated with rust resistance in other varieties (8) which are related to Redman. The present findings also agree in part with Campbell's proposal (2) that complementary dominant factors in Redman condition resistance to this race and verify his suggestion that chromosome III is involved.

Although one susceptible monosomic plant was observed in each of lines I and XX, the other data from these two lines indicate that these chromosomes do not carry genes for rust resistance. Eleven of the 12 monosomic plants in line I and 4 of the 5 monosomic plants in line XX were resistant. One possible explanation for these discrepancies is that of "univalent shift", as postulated by Person (5), whereby one monosomic line might change to another in plants exhibiting slight asynapsis. Throughout this study precautions were taken against such a shift by discarding monosomic parent plants showing a high frequency of univalents, but the possibility of such a change still exists.

Chromosomes IX and XVII of Redman each carry a dominant factor for awn suppression. In all cases, plants deficient for either of these Redman chromosomes were as fully awned as Prelude, while normal plants were awnletted like the Redman parent. Since the absence of either of the suppressor factors allowed awn expression, the action here also must be

TABLE 1.—CLASSIFICATION OF PARENTAL AND F_1 POPULATIONS FOR RUST REACTION AND AWN EXPRESSION

Parental variety or chromosome involved in F_1	Total number of plants	Rust Reaction				Awn Expression			
		Disomics		Monosomics		Disomics		Monosomics	
		No. Resist.	No. Susc.	No. Resist.	No. Susc.	No. Awnletted	No. Awned	No. Awnletted	No. Awned
Prelude	10		10				10		
Redman	9	9				9			
I	16	4		11	1*	4		12	
II	6	2		4		2		4	
III	11	2		0	9	2		9	
IV	5	3		2		3		2	
V	13	6		7		6		7	
VI	11	7		4		7		4	
VII	13	2		11		2		11	
VIII	9	4		0	5	4		5	
IX	11	4		7		4		0	7
X	13	5		8		5		8	
XI	4	2		2		2		2	
XII	12	6		6		6		6	
XIII	24	13		0	11	13		11	
XIV	16	6		10		6		10	
XV	12	6		6		6		6	
XVI	6	2		4		2		4	
XVII	5	2		3		2		0	3
XVIII	10	4		6		4		6	
XIX	22	2		20		2		20	
XX	8	3		4	1*	3		5	
XXI	21	5		16		5		12	4**
	248	90		131	27	90		144	14

* No specific explanation can be advanced for this single plant discrepancy.
** All 4 plants were progeny of the same monosomic plant and 2 are known to be heterozygous for a reciprocal translocation.

complementary. O'Mara (4) found that Marquis, which is predominant in the parentage of Redman, has a single awn inhibitor located on chromosome IX. No previous report of a gene affecting awn expression on chromosome XVII is known. The data from chromosome XXI are conflicting in that both awnletted and awned monosomic plants were present. However, 12 of the 16 monosomics were awnletted and at least 2 of the 4 awned ones were heterozygous for a reciprocal translocation. Furthermore, all 4 awned plants were progeny of the same Redman monosomic plant, which would indicate that some aberration was present in this parent. Therefore, the evidence strongly suggests that chromosome XXI of Redman does not carry a suppressor gene, and the conflicting data probably can be safely disregarded in this study.

In previous genetic studies to determine chromosomes carrying factors for rust resistance and awning the original Chinese Spring monosomics have been used, and hence these studies have had the Chinese Spring genetic background in common. The use of the Redman monosomics constitutes the first departure from this practice. Since the results of this investigation conform closely with earlier findings, it would appear that the genetic background of the monosomic tester variety does not have a significant effect.

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FACTORS INFLUENCING INFESTATION AND INJURY OF RUTABAGAS BY ROOT MAGGOTS (DIPTERA:ANTHOMYIIDAE) IN PRINCE EDWARD ISLAND. I. FIELD STUDIES[1]

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ABSTRACT

Examinations of more than 100 rutabaga fields per year, from 1951 to 1955, showed that *Hylemya brassicae* (Bouché) was the only species of root maggot that injured rutabagas in Prince Edward Island. *H. liturata* (Mg.) (=*H. trichodactyla* (Rond.)), *H. cilicrura* (Rond.), and *Muscina stabulans* (Fall.) were associated with *H. brassicae* but did not injure the roots. *H. brassicae* flies [note Whitcomb (12) for illustrations] began to emerge from overwintered puparia early in June in sandy soil areas and late in July in clay loam areas. Heavy texture and high moisture content of soils were closely correlated with the delay in emergence. The flies laid their eggs near rutabaga plants, usually in crevices in the soil, and upon hatching the larvae entered and fed on the roots. Larvae generally entered the roots at a depth of one inch or more below the surface of the soil. In general, early-planted rutabagas in sandy soil areas and late plantings in clay loam areas were severely damaged by larvae of *H. brassicae*, whereas late plantings in sandy areas and early plantings in clay loam areas were lightly infested. However, in the sandy soil areas where *all* of the rutabaga crops were harvested early in August damage was relatively light. Also, fields isolated by other rutabaga fields from sources of infestation such as storage bins and infested rutabaga crops, of either the current or the previous season, were usually slightly damaged. Use of barnyard manure increased *H. brassicae* infestations early in the season but did not significantly influence the damage caused during the whole growing season.

INTRODUCTION

Rutabagas (swede turnips) are grown in Prince Edward Island on a comparatively large scale for export and for livestock feed. The rutabagas must be of high quality to meet export requirements and competition from other areas. Damage by root maggots is the most important factor restricting the production of high-quality rutabagas in the province. Between 1945 and 1955 the acreage of rutabagas grown in this province dropped from approximately 12,000 acres to less than 7,000 acres, and there was a corresponding decrease from about 1,600,000 bushels to slightly less than 1,000,000 bushels shipped annually from the Province as table stock. Much of the decrease occurred because root maggot injury (note Figures 4 and 5) has made it difficult for growers to produce a marketable crop.

Many investigations on root maggots attacking cruciferous crops have been reported in Canada, the United States, and Europe. However, most of these have been devoted to studies on cabbage and cauliflower, and it is only in recent years that special attention has been directed towards rutabagas.

This is a report on field studies of maggot infestations in rutabagas from 1951 to 1955 in Prince Edward Island, especially on the effects of soil and climate on infestations of the cabbage maggot, *Hylemya brassicae* (Bouché).

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METHODS

More than 100 rutabaga fields in representative localities throughout the Province were observed each year from 1951 to 1955. The fields were examined at least three times during the growing season to determine: species of maggots present in and around the roots, numbers of injured plants and degree of injury per plant in different sections of fields, and period of attack by each generation. Acreage, variety, date or dates of planting, crop rotation, and use of barnyard manure and commercial fertilizer were recorded for each field. Factors such as distance of a field from storage-bins and from rutabaga fields of the current or the previous season, and cultivation of the previous year's rutabaga field after the crop was harvested, were also considered.

General field observations in 1951 indicated that infestations did not occur at the same time throughout the Province. To determine when the first flies emerged and the duration of emergence, 6 pyramidal cages were placed in each of 11 fields in different areas in the spring of 1952. These fields, representative of the different soil areas of the province, had been heavily infested with *H. brassicae* in 1951. The cages, with cheesecloth fastened to the inside of a wooden framework, were 5 feet square at the base and 2½ feet high. To eliminate the possibility that the cages would have some influence on the temperature and moisture content of the soil under them, they were moved to a new site every 3 or 4 days. The emerging flies were collected at 3-day intervals and identified with the aid of Brooks' (3) key. Similar tests were conducted from 1953 to 1955.

Dates of first attack by first-generation larvae of *H. brassicae* and periods of peak abundance of larvae and adults in different areas were estimated from larval collections and from data obtained with the emergence cages. To determine when attacks by larvae of the second generation started, infested plants were collected from representative fields when the attack by the first generation started in each area. These plants were transplanted in non-infested soil under cages 5 feet square at the base and 2½ feet high. When flies emerged they were transferred to new cages that contained non-infested rutabagas and second-generation larvae were allowed to develop in these plants. The same procedure was followed to determine the time of first attack by larvae of a third generation. The duration of attacks by each generation was determined by comparing the above data with the periods of peak abundance of larvae in each area.

Soil texture was determined from samples collected in rutabaga fields throughout the province each fall from 1951 to 1955. Samples consisted of 20 random sub-samples per field taken to a depth of 6 inches with a ¾-inch soil sampling tube. The soils were tested by the Bouyoucos mechanical analysis method (1), and classed as sandy, loam, and clay loam according to the system used by Lyon and Buckman (6). According to Whiteside (13), the sandy soils correspond approximately to Kildare sandy loam, the loam to Charlottetown fine sandy loam, and the clay loam to Queen's clay loam. The results of these tests were compared with the first appearance of larvae of *H. brassicae* in the following year's rutabaga field that adjoined each soil-tested field.

Soil moisture tests were made on samples collected at weekly intervals during June and July, 1955, from 19 infested fields in representative areas of the province. Each sample, consisting of 20 random sub-samples from a 1-acre area in each field, was collected by taking $\frac{3}{4}$-inch cores of soil to a depth of 3 inches, discarding the upper 2 inches, and retaining the remaining 1 inch for analysis. The moisture content was determined by oven-drying the samples at 105°-110° C. The results were compared with the time of first appearance of larvae of *H. brassicae* in the fields.

RESULTS

Species Found, and Abundance

Although larvae of *H. brassicae*, *H. liturata*, *H. cilicrura*, and *Muscina stabulans* (Fall.) were all associated with rutabagas, the cabbage maggot was the only one causing primary injury. Larvae of this species were present in the roots from late May to December and all of the fields examined from 1951 to 1955 were infested, 2 to 100 per cent of the plants in a field being attacked. Large numbers of eggs of *H. liturata* were deposited around rutabagas, but larvae from these eggs were not found in or near the plants unless larvae of *H. brassicae* were already in the roots. During the period of greatest abundance, the average numbers of eggs and larvae of the two species collected from 10 plants in each of four fields in each of three representative areas of the Province were:—

	Clay loam area	Sandy loam area, A	Sandy loam area, B
Eggs, July 20, *H. brassicae*	3	62	1
H. liturata	72	79	203
Eggs, July 28, *H. brassicae*	56	58	0
H. liturata	12	34	63
Larvae, Aug. 12, *H. brassicae*	47	76	0
H. liturata	0	167	0

In the clay loam area, eggs of *H. liturata* were laid before those of *H. brassicae* and no larvae of *H. liturata* were found in or near the roots. In the sandy loam area (A), eggs of both *H. brassicae* and *H. liturata* were abundant at the same time and large numbers of larvae of *H. liturata* developed around the rutabagas. In the sandy loam area (B), eggs of *H. liturata* were abundant but those of *H. brassicae* were few and no larvae of either species developed in or near the roots. Observations indicated that the larvae of *H. liturata* fed on refuse or rotting material caused by *H. brassicae* and not on healthy root tissue. In two fields with very severe injury, the average numbers of larvae in 10 plants selected at random were:—

	H. brassicae	*H. liturata*
In wet soil near rutabagas	11	234
Inside rutabagas	109	8

Studies on emergence showed that large numbers of *H. liturata* overwintered in rutabaga fields, especially in sandy soil areas; in a field that was heavily infested with *H. brassicae* the previous season, as many as 750 *H. liturata* flies were captured in cages covering an area of 300 square feet. Also, field sweeps during the growing season indicated that *H. liturata* flies were often more abundant than those of *H. brassicae*; during August the ratio was as high as 50 to 1 in some fields. *H. liturata* emerged from overwintered puparia in all areas of the Province between late May and early July, the peak occurring during the third week of June.

Larvae of *H. cilicrura* were rarely found in rutabaga fields, and they comprised less than 1 per cent of the total numbers of root maggots found in or near rutabagas.

M. stabulans was found only in fields near farm buildings during late May and June. Egg counts were as high as 34 per plant per day during early June in a few low wet fields, but severe injury by the larvae was noted in only one field; this occurred during continuous wet weather.

Observations on Infestations of H. brassicae

First-generation larvae of *H. brassicae* caused the most injury. Small plants were either killed or distorted, but if the plants were large and soil moisture was abundant when the attack occurred, larvae fed near the surface and seldom tunnelled into the roots. Injury from the first attack caused the roots to become hard and woody, and attacks occurring later in the season caused only slight damage.

In sandy or sandy loam areas, most early-planted rutabaga crops were heavily infested (note Figure 3); the largest numbers of maggots occurred on farms where early and late plantings were grown in the same field, or where part of the early-planted crop was left in the field throughout the growing season. However, on farms where *all* the rutabagas were harvested early, or where only late-planted crops were grown, maggot injury was slight.

In loam or clay loam areas, early-sown crops were relatively free of injury if harvested in July or early August, whereas late plantings were in general severely damaged. If early and late plantings were in the same field and both were left in the field until October, only the late planting was attacked; however, if no late-planted crop was grown, the early planting was attacked in August and September.

Dry weather was the most important single factor that reduced the numbers of maggots in a rutabaga crop. During wet weather an increase in the number of adults in a field was always closely followed by an increase in the number of larvae. However, if a dry period started while adult populations were increasing, the numbers of larvae began to decrease almost immediately; after about 6 or 7 days, only second- or third-instar larvae were present; and after 10 days to 2 weeks, the maggots practically disappeared from the roots, even though the adults were still present in the fields and egg counts had decreased only slightly. The eggs and young larvae apparently died before gaining entry into the roots.

Observations on the influence of seasonal moisture conditions on infestations during the study showed the following trends: In 1951, the growing season was relatively wet and maggot injury was severe; there was a

TABLE 1.—AVERAGE NUMBERS OF *H. brassicae* FLIES EMERGING FROM OVERWINTERED PUPARIA DURING 10-DAY INTERVALS FROM 12 CAGED AREAS TOTALLING 300 SQ. FT. PER FIELD FOR THREE TYPES OF SOIL IN REPRESENTATIVE LOCALITIES OF PRINCE EDWARD ISLAND, 1952–1955*

Number of fields	Soil	June			July			August			September	
		1–10	11–20	21–30	1–10	11–20	21–31	1–10	11–20	21–31	1–10	11–20
						1952						
4	Sandy		12	81	169	41	4					
4	Loam				7	12	96	30	20	14		
3	Clay loam						6	30	6	4	14	
						1953						
5	Sandy		6	14	21	19	17	10				
4	Loam				10	24	18	13	8			
2	Clay loam						12	42	38	16		
						1954						
2	Sandy	14	23	27	39	86	3	2				
2	Loam			18	45	56	84	40	22	14		
2	Clay loam					37	46	157	174	68	14	4
						1955						
2	Sandy		17	46	64	36	61	34	2			
2	Loam			28	68	200	240	85	47	10		
1	Clay loam						4	49	51	8	11	6

* Each field was known to have been heavily infested the previous season.

marked increase in the numbers of larvae during the growing season and large numbers of puparia overwintered. In 1952, which was dry throughout, large numbers of flies emerged from the overwintered puparia but the numbers of both adults and larvae decreased steadily during the summer; injury at harvest was slight, and the numbers overwintering were small. In 1953 and 1954, which were wet throughout, there was a steady increase in the numbers of larvae, and the numbers of puparia overwintering were large. In 1954, injury in the fall was very severe in almost all rutabaga fields, and even in the late-planted crops in sandy soil areas more than 40 per cent of the plants in some fields were killed by maggots. In 1955, large numbers of maggots caused very severe injury in early-planted crops in the sandy areas during wet weather in June and early July. However, very dry weather prevailed throughout July, August, and September; the numbers of maggots decreased sharply, and only slight injury was caused in late-planted crops in sandy areas and in all rutabaga crops in loam or clay loam areas.

Rutabaga crops planted within about 100 yards of storage buildings or infested fields of the previous season were generally 100 per cent infested and had moderate to very severe damage, whereas fields isolated from these sources of infestation by other rutabaga fields usually were lightly infested. A large number of field observations showed that the flies travelled mainly in the direction of the prevailing winds (from southwest to northeast). Along the southern shoreline, rutabaga fields south of sources of infestation always had much lighter injury than those north of the sources. If two fields were about 100 feet apart in a direct line south of an infestation source, the one nearer the source was infested, whereas the one farther to the south was practically free of injury. Conversely, if two fields were in a line north of the source of infestation, both were severely damaged, although the one nearer the source had more injury.

Applications of barnyard manure did not influence injury by maggots appreciably. During the early part of the growing season, 95 to 100 per cent of the rutabagas in manured sections of fields were infested as compared with less than 50 per cent in unmanured sections, but as the season progressed all sections became equally infested. At harvest, no difference in the extent of injury was detected between manured and unmanured areas.

Emergence of Adults from Overwintered Puparia

Table 1 shows that *H. brassicae* began emerging during the second week of June in the sandy soil areas, and from mid- to late July in the clay loam areas. In areas of intermediate soil, the time of first emergence varied accordingly. The period of emergence was approximately 6 weeks in all the areas studied.

Influence of Soil Texture and Moisture

The studies on soil texture showed that there was a close relationship between soil texture and time of maggot attack (Figure 1). The correlation coefficients (negative for each year) were: .85 for 1952, .92 for 1953, .93 for 1954, and .82 for 1955, all being significant at the 1 per cent level. The first attack by *H. brassicae* larvae began more than 7 weeks later in the heaviest clay loam areas than in the lightest sandy areas.

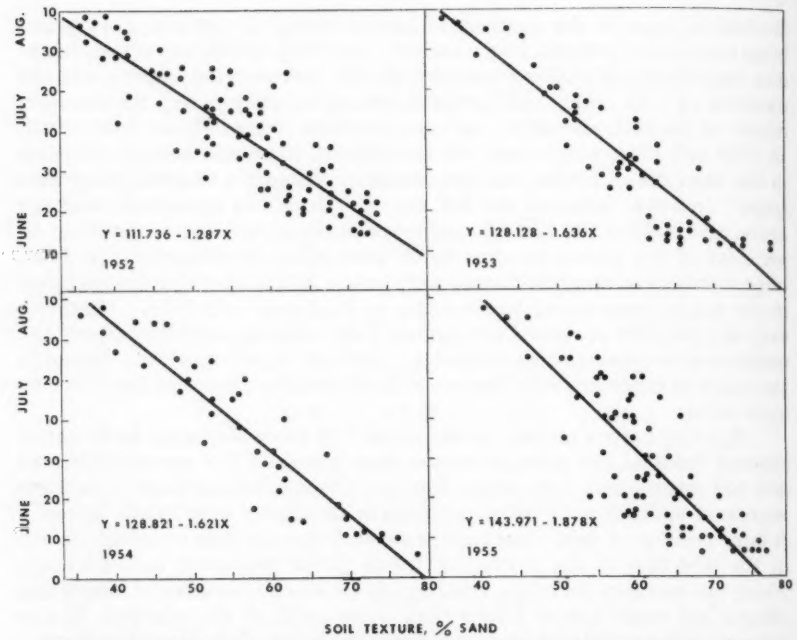

FIGURE 1. Dates of first appearance of *H. brassicae* larvae in rutabagas in relation to percentage of sand in soils from representative fields in Prince Edward Island, 1952 to 1955. Each plotted point represents one field.

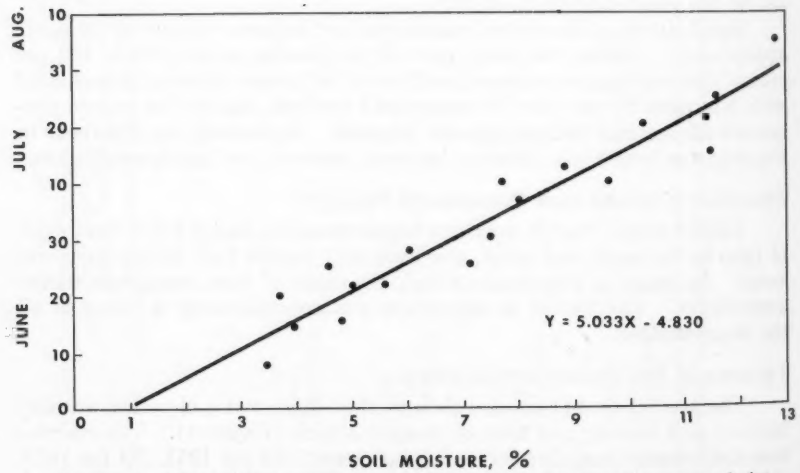

FIGURE 2. Date of first appearance of *H. brassicae* larvae in rutabagas in relation to percentage of soil moisture in representative fields in Prince Edward Island, 1955. Each plotted point represents one field, the percentage moisture being the mean of weekly values determined during June and July.

Figure 2 shows the relation between percentage of moisture in the soil and the time of first maggot attack. The coefficient of correlation was .96, which was significant at the 1 per cent level.

Influence of Predacious Insects

The dipterous predator Coenosia tigrina (Fall.) was first noted in Prince Edward Island in 1951, when puparia were taken to the greenhouse in soil collected from fields infested by the cabbage maggot. Puparia overwintered in rutabaga fields and the flies emerged at about the same time as *H. brassicae*. In cages the flies destroyed an average of up to 8 *H. brassicae* adults per day but, as observed by Perron *et al.* (8), fewer were killed per day as the predators grew older. Field collections made with sweep nets indicated that *C. tigrina* was abundant throughout the province, and was sometimes more numerous in grazed pasture fields than in rutabaga fields. The flies were observed killing species of Hymenoptera as well as *H. brassicae*, *H. liturata*, and other Diptera.

Staphylinids, *Aleochara bilineata* (Gyll.) and unidentified species, were present in all maggot-infested rutabaga fields, and in many instances they were observed destroying larvae of *H. brassicae* inside rutabagas. Carabid beetles were observed destroying eggs of *H. brassicae*.

Other Factors

General field observations, soil texture studies, and studies with the emergence cages showed that larvae of two generations of *H. brassicae* occurred in the sandy soil areas and of one generation in the clay loam areas (Figure 3). Also, field-collected larvae from the different areas reared under cages in the fields gave rise to a small number of larvae of a third generation in the sandy soil areas and of a second generation in the clay loam areas.

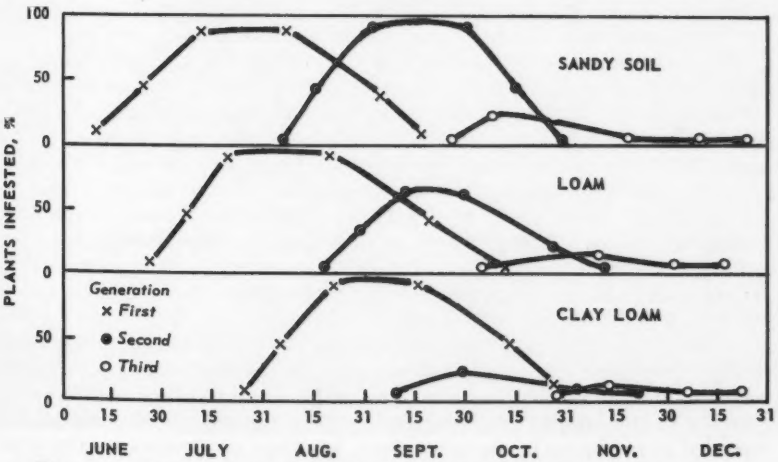

FIGURE 3. Percentages of plants infested during the season by larvae of various generations of *H. brassicae* in areas of Prince Edward Island according to the type of soil. Mean values are given of counts from approximately 100 heavily infested rutabaga fields in each soil area from 1951 to 1955. The generations were separated with records from rearings in cages in each of the areas.

Although these later generations were not distinguished in the field, severe internal tunnelling of roots harvested in late October sometimes occurred, and the larvae causing this injury were probably of these generations.

Generally a field of rutabagas was attacked by only one generation of maggots. If a crop was planted in late April or early May in a sandy soil area, it was usually harvested in August, before the second generation of larvae became abundant. However, even if the rutabagas were left in the field until October, second-generation maggots were few and injury was slight; injury by first-generation larvae caused the roots to become hard and woody and a high percentage of the young larvae from the second generation probably desiccated or were destroyed by predators before they were able to enter the roots.

Plants attached at different stages of development suffered varying degrees of injury (Figure 4). Injury to young plants resulted in distortion, whereas injury to mature roots consisted mainly of surface scarring. Internal tunnelling seldom occurred until after the rutabagas were removed from the soil. The extent of this tunnelling depended on the stage of development of the larvae at the time the rutabagas were harvested (Figure 5), and severe injury was found only in roots that were harvested when they contained first- and second-instar larvae. During prolonged periods of dry weather, larvae sometimes tunnelled inward and pupated inside the roots, but under more moist conditions they fed in the surface layers and entered the soil to pupate.

DISCUSSION

H. liturata has been reported by early workers as a pest of cruciferous crops, and Brooks (3) states that it may cause root damage under certain conditions but it normally forms only about 10 per cent of the root maggot population. The present study shows that this species may form as high as 70 per cent of the maggot population, but its abundance depends on *H. brassicae* being already present in the roots. In clay loam areas the first generation of *H. brassicae* appears later than that of *H. liturata*, and larvae of *H. liturata* are therefore rarely found in these areas. In sandy areas the two species appear at approximately the same time and very large numbers of larvae of *H. liturata* may be present around rutabagas. However, the larvae are rarely found inside the roots and there is no indication that they cause injury to the plants. Since *H. cilicrura* and *M. stabulans* are the only other species of root maggots found associated with rutabagas, and they are present in small numbers, it is concluded that *H. brassicae* is the only species of root maggot causing economic injury to rutabagas in Prince Edward Island.

Predacious insects, such as Staphylinids, *C. tigrina*, and carabid beetles, destroy different stages of *H. brassicae*, and in this study an increase in the numbers of larvae of *H. brassicae* in a field was always accompanied by an increase in the numbers of predators. Species of rove beetles have been recorded as predators by many workers, but their importance in control of root maggots has not been ascertained. *C. tigrina*, as reported by Perron *et al.* (8), was first recorded in Canada in 1943 and is reported as being predacious mainly on small species of Diptera and some Hemiptera. It has been observed by the writer to destroy also species of Hymenoptera that

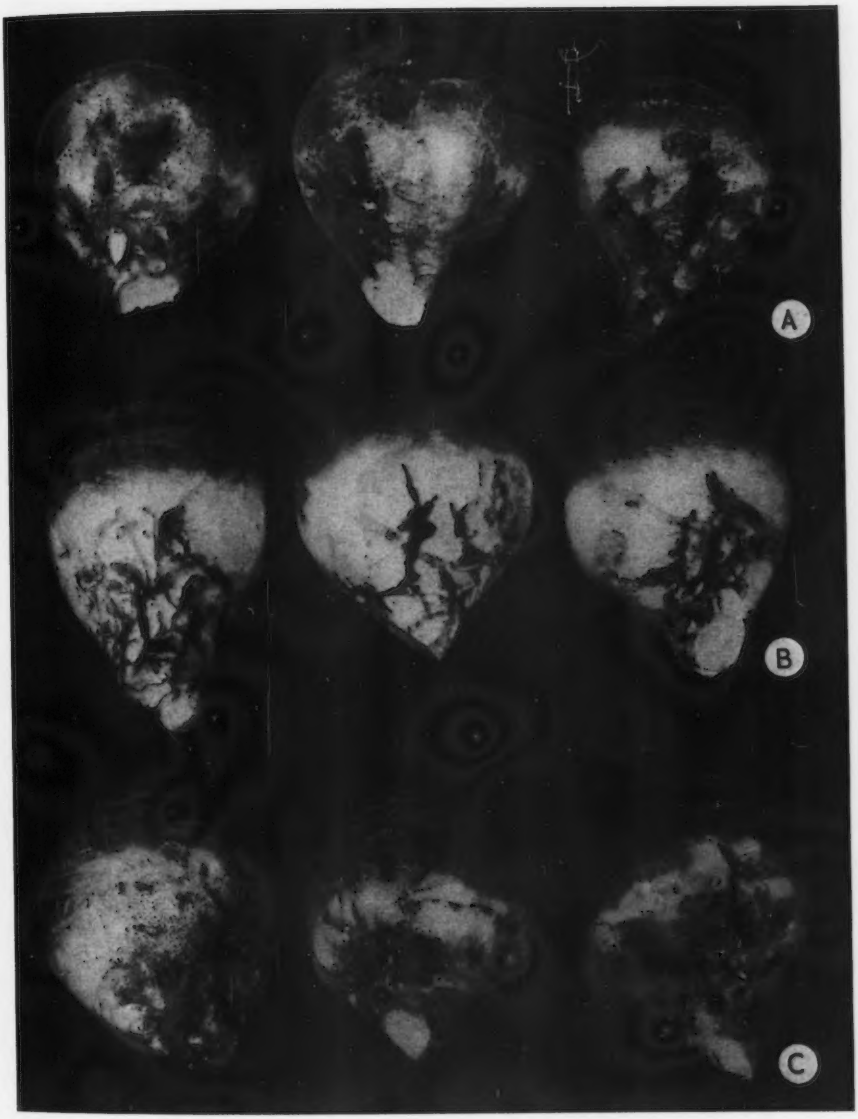

FIGURE 4. Rutabagas injured at different stages of development by larvae of *H. brassicae*. A—Distortion of roots from injury to young plants. B.—Surface scarring from injury to nearly mature plants. C.—Distortion and surface scarring from early and late injury.

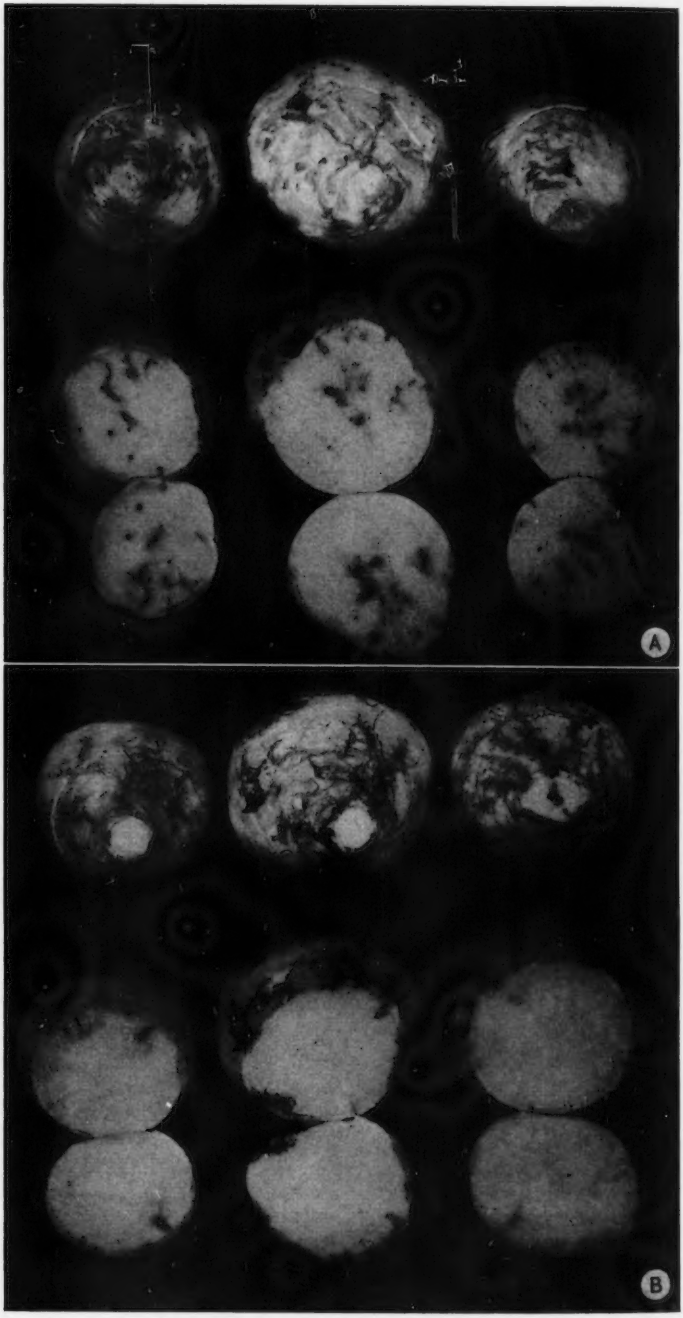

FIGURE 5. External and internal appearance of injured rutabagas after storage for 6 days at room temperature. A—Only first-instar larvae present at harvest. B—Only second- or third-instar larvae present at harvest.

were more than twice its size. Adults of *C. tigrina* are sometimes far more abundant in pasture fields than in neighbouring rutabaga fields, and the predator apparently moves to rutabaga fields only when the latter contain an abundance of host insects.

One to five generations of *H. brassicae* have been reported from different parts of the world (2-5, 7, 9-12). In warmer areas with long growing seasons, a larger number of generations may be produced than in more temperate zones. However, within a country or an area having a relatively uniform climate, the soil type may influence the number of generations produced. In Prince Edward Island there are fewer generations in the clay loam areas than in the sandy areas and these result, either directly or indirectly, from the influence of soil texture and soil moisture.

The close relationship between the amount of sand in the soil in an area and the time of first attack by maggots (Figures 1 and 3) may be used by farmers to aid in reducing maggot injury. In the light sandy areas where the first-generation attack occurs mainly in late June and July, the growers as a group may follow one of two systems of cropping: they may grow either only early-planted rutabagas (sown in late April or early May) and harvest the whole crop in early August, or only late-planted rutabagas (sown in late June or early July). Harvesting all of the crop in August removes maggots that are in the roots, and most flies emerging from puparia already formed in the field would be unlikely to find suitable host plants. If all farmers in an area followed the same practice each year, the root maggot populations would be kept at a minimum. Infestations also tend to be light in sandy areas where only late-planted crops are grown. The first-generation attack is avoided and, since movement of flies from one field to another during the season is limited, heavy infestations do not develop late in the season.

The heaviest infestations in sandy soil areas occur on farms where early- and late-planted crops are grown in the same field. Flies of the overwintered generation produce larvae that infest early plantings, and adults from the first generation emerging in August and September tend to move into adjoining late-planted crops. Mortality of eggs and young larvae is lower around the more succulent roots of the late planting than around the mature, hardened roots of the early planting; maggot populations may be maintained or even increased during the growing season and overwintering populations each year are high.

In clay loam areas where flies emerge from overwintered puparia in late July and August, farmers may avoid severe maggot injury by planting the crops in late April or early May and harvesting in early August. Since crops planted later and harvested in October are exposed to attack during the whole period of emergence of flies from overwintered puparia, they are usually heavily infested.

The general practice for producing rutabagas for table stock in Prince Edward Island is to plant in late April or early May and harvest in July or early August, or to plant in late June or early July and harvest in late October. It is, therefore, possible for larvae of only one generation of *H. brassicae* to attack a particular crop. The above cultural methods to keep maggot infestations at a minimum may be followed by farmers in

more isolated areas. However, there is a limited market for rutabagas harvested in August and, especially in areas where many rutabagas are grown, most growers must resort to chemical methods of control for satisfactory protection from damage.

ACKNOWLEDGEMENTS

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DETERMINATION OF THE NUMBER AND DOMINANCE RELATIONSHIPS OF GENES ON SUBSTITUTED CHROMOSOMES IN COMMON WHEAT, *TRITICUM AESTIVUM* L.[1]

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ABSTRACT

A study of awning, culm colour and reaction to pseudo-black chaff in crosses of seven different substitution lines with Chinese Spring wheat was made to illustrate a method that permits the determination of the number and dominance relationships of genes on a substituted chromosome governing a particular character. F_2 and F_3 results in each of the seven crosses showed that each of the substituted chromosomes carries one gene affecting the character under investigation. On the basis of F_1 results, genes for apical awning on chromosome III of Thatcher and Timstein and IV, XII and XXI of Thatcher were found to be recessive; Hope possesses a dominant gene for purple culm colour on chromosome VII and a recessive gene for susceptibility to pseudo-black chaff on chromosome III. Chinese contains alternative alleles for all these genes. A study such as outlined in this report must supplement the study of substitution lines to provide a complete genetic analysis of the character under investigation. The reasons for a supplementary study as well as the advantages and disadvantages of the substitution method in comparison with other methods of analysis are discussed.

INTRODUCTION

The chromosome substitution method has been found to be valuable in analysing the genetic basis of quantitative and qualitative characters in common wheat, *Triticum aestivum* L.* (3, 7). It permits the association of genes with specific chromosomes but does not allow the determination of dominance relationships, nor does it permit the determination of the number of genes a chromosome carries affecting a specific character.

This report deals with a method that permits the determination of the number, and also the dominance relationships, of genes on a substituted chromosome governing a particular character. The principle of the method is that, when the substitution line is crossed with the recipient variety, the resulting hybrid is heterozygous for only one chromosome pair and segregation will only be for genes on that pair. Three qualitative characters are used to illustrate the method in seven different crosses.

REVIEW OF LITERATURE

The results of a study at the University of Alberta reported by Kuspira and Unrau (3) using the substitution method for genetic analysis indicate that the substitution of certain chromosomes of Thatcher, Hope and Timstein into Chinese** produced rather clear-cut effects on awning, earliness, height, lodging, yield, etc. Sears, Loegering and Rodenhiser (7), using this method, have also demonstrated an effect of a number of Hope, Thatcher,

* Synonymous with *T. vulgare* Vill., until recently the commonly accepted botanical name of the species although *T. aestivum* has priority since it was the original Linnean designation.

** Chinese Spring.

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Timstein and Red Egyptian chromosomes on stem rust resistance. In these studies, however, no data have been obtained regarding the number of genes per chromosome affecting the character concerned.

Of the characters to be reported on, awning has been most extensively studied. According to Sears (5), Watkins and Ellerton (9) and Kuspira and Unrau (3) numerous genes, whose presence in chromosomes II, III, IV, VIII, IX, X, XII and XX has been confirmed, govern this character. Only a few studies have been made on the mode of inheritance of culm colour (8, 10), both of which indicate monogenic control of this character. Least studied is resistance to black-chaff and pseudo-black chaff (1, 2, 4), the mode of inheritance of which has not heretofore been determined.

MATERIALS AND METHODS

Seven different substitution lines, Chinese (2 III Thatcher)*, Chinese (2 IV Thatcher), Chinese (2 XII Thatcher), Chinese (2 XXI Thatcher), Chinese (2 III Hope), Chinese (2 VII Hope) and Chinese (2 III Timstein), each differing from Chinese in a gene or genes governing the inheritance of a particular qualitative character, were used to determine the number of genes carried by a substituted chromosome.

The method of producing substitution lines has been described elsewhere (3, 6). The initial step consists of crossing nullisomic or monosomic plants of the recipient variety (deficient for the chromosome concerned) as female with the variety to be analysed. Monosomic plants are selected from the F_1 and backcrossed as males to the original nullisomics or monosomics. This process is repeated with the first and subsequent backcross progenies. After a sufficient number of backcrosses the genotype of the recipient parent will be reconstituted for each of the 20 pairs of chromosomes not involved in the transfer. The chromosome being transferred (the univalent in the F_1 and backcross monosomics) will be genetically identical with that of the donor parent, having had no opportunity to pair and cross over with a homologous chromosome. At the end of the backcrossing program the monosomic plants are allowed to self-pollinate. Approximately one-fourth of the resulting progeny are disomic, having 20 pairs of reconstituted chromosomes of the recipient parent plus one pair of unchanged chromosomes for the donor parent.

The monosomics used as the recurrent parent may have a normal whole chromosome, a telocentric chromosome or an isochromosome in the univalent state. When monosomics containing normal whole chromosomes are used the male parent must be allowed to self-pollinate between successive generations; otherwise there is the possibility of obtaining monosomics deriving their univalents from the female instead of the male parent. If, however, monosomics having telocentric chromosomes or isochromosomes are used selfing is not necessary because these chromosomes can be cytologically distinguished from the normal whole chromosomes.

Chinese was reciprocally crossed with each of the substitution lines to eliminate the cytoplasm as a factor in conditioning any of the characters studied. The plants used for crossing were cytologically analysed to make certain that all plants used were normal with 21 bivalents.

* The designation "Chinese (2 III Thatcher)" indicates a line of Chinese Spring disomic for chromosome III of Thatcher.

If heterozygosity exists in substitution lines there is the possibility of using plants for crossing that have donor parent genes on chromosomes other than the one transferred. Crosses between such plants and the recipient parent would produce results leading to the false assumption that these genes are located on the substituted chromosome. To minimize this danger, the substitution lines used had been backcrossed to Chinese seven times during the process of their development, before being used in this study.

F_1, F_2, and F_3 plants were classified for awn-type, culm colour and reaction to pseudo-black chaff. A genetic analysis of the F_2 and F_3 populations was then carried out to determine the mode of inheritance of the above-mentioned characters. The number of genes governing awning was studied in the crosses Chinese × Chinese (2 III Thatcher), Chinese × Chinese (2 IV Thatcher), Chinese × Chinese (2 XII Thatcher), Chinese × Chinese (2 XXI Thatcher) and Chinese × Chinese (2 III Timstein). Culm colour was investigated in the Chinese × Chinese (2 VII Hope) cross and pseudo-black chaff in the Chinese × Chinese (2 III Hope) cross.

A description of the substitution lines and Chinese with respect to the three characters studied is given in Table 1.

In the analysis of awning only two classes, depending on the extent of awn development, were used: (1) awnless, and (2) apically awned. In the latter class short awnlets were present near the base of the spike and the length of awns increased towards the apex, the upper half usually being almost fully awned.

The development of culm colour is influenced considerably by environmental conditions, which are often extremely variable in the field and at times prevent the full expression of the character. In the greenhouse, conditions may be controlled to allow 100 per cent or nearly 100 per cent expressivity of the character. Two classes of plants were recognized in the F_2 and F_3, those having purple culms, as in the substitution line, and those having white culms, as in Chinese. The F_1's and F_2's were grown in the greenhouse; F_3's were analysed in the field.

TABLE 1.—DESCRIPTION OF THE SUBSTITUTION LINES AND CHINESE WITH RESPECT TO THE THREE CHARACTERS STUDIED

Substitution line or variety	Character		
	Awning	Culm colour	Reaction to pseudo-black chaff
Chinese (2 III Thatcher)	Apically awned	White	Resistant
Chinese (2 IV Thatcher)	Apically awned	White	Resistant
Chinese (2 XII Thatcher)	Apically awned	White	Resistant
Chinese (2 XXI Thatcher)	Apically awned	White	Resistant
Chinese (2 III Hope)	Awnless	White	Susceptible
Chinese (2 VII Hope)	Awnless	Purple	Resistant
Chinese (2 III Timstein)	Apically awned	White	Resistant
Chinese	Awnless	White	Resistant

Pseudo-black chaff affects various parts of the spike as well as any or all parts of the culm. Any plant having a portion of the spike or culm affected was classed as diseased and plants free of discoloration and blackening were classed as non-diseased.

The Chi-Square test for goodness of fit was used in analysing the results.

RESULTS

The F_1, F_2 and F_3 results are presented in Tables 2, 3 and 4 respectively.

F_1 results

The F_1's of the five crosses involving substitution lines Chinese (2 III Thatcher), Chinese (2 IV Thatcher), Chinese (2 XII Thatcher), Chinese (2 XXI Thatcher) and Chinese (2 III Timstein) were all awnless, showing that the genes for awnlessness on Chinese chromosomes III, IV, XII and XXI are dominant and that the donor varieties carry the recessive alleles.

The Chinese × Chinese (2 VII Hope) F_1's all possessed purple culms. These results substantiate previous findings (8, 10) that the gene (or genes) for purple pigment formation is dominant. Chinese and other white-culmed varieties must possess the recessive allele (or alleles) on chromosome VII.

No pseudo-black chaff was detected in F_1's of the Chinese × Chinese (2 III Hope) cross. This indicates that the resistance of Chinese is caused by a dominant gene (or genes) on chromosome III, the recessive allele (or alleles) for susceptibility being present in Hope.

F_2 and F_3 results

The reasonably good fit of each of the F_2 populations to a 3:1 ratio for awnless:apically awned in Chinese × Chinese (2 III Thatcher), Chinese × Chinese (2 IV Thatcher), Chinese × Chinese (2 XII Thatcher), Chinese × Chinese (2 XXI Thatcher) and Chinese × Chinese (2 III Timstein) indicates that each of the substituted chromosomes carries one recessive gene conditioning awning. This finding is corroborated by the F_3 breeding behaviour of F_2 plants. All awnless F_2 plants either bred true, or segregated, whereas all awned F_2 plants bred true in the F_3.

TABLE 2.—PHENOTYPIC CHARACTERS OF THE F_1'S OF CROSSES FOR THEIR RESPECTIVE CHARACTERS

Cross	Phenotype		
	Awning	Culm colour	Reaction to pseudo-black chaff
Chinese × Chinese (2 III Thatcher)	Awnless	White	Resistant
Chinese × Chinese (2 IV Thatcher)	Awnless	White	Resistant
Chinese × Chinese (2 XII Thatcher)	Awnless	White	Resistant
Chinese × Chinese (2 XXI Thatcher)	Awnless	White	Resistant
Chinese × Chinese (2 III Hope)	Awnless	White	Resistant
Chinese × Chinese (2 VII Hope)	Awnless	Purple	Resistant
Chinese × Chinese (2 III Timstein)	Awnless	White	Resistant

TABLE 3.—SEGREGATION FOR AWN TYPES, CULM COLOUR AND REACTION TO PSEUDO-BLACK CHAFF IN F_2 POPULATIONS OF EACH OF THE SEVEN CROSSES

Cross	Awning		Culm colour		Reaction to pseudo-black chaff		X^2 for 3:1 ratio	P
	Awnless	Apically awned	Purple	White	Resistant	Susceptible		
Chinese × Chinese (2 III Thatcher)	147	43	—	—	—	—	0.57	0.30 – 0.50
Chinese × Chinese (2 IV Thatcher)	126	37	—	—	—	—	0.46	0.30 – 0.50
Chinese × Chinese (2 XII Thatcher)	111	43	—	—	—	—	0.70	0.30 – 0.50
Chinese × Chinese (2 XXI Thatcher)	119	42	—	—	—	—	0.10	0.50 – 0.95
Chinese × Chinese (2 III Hope)	—	—	—	—	138	40	0.61	0.30 – 0.50
Chinese × Chinese (2 VII Hope)	—	—	184	68	—	—	0.53	0.30 – 0.50
Chinese × Chinese (2 III Timstein)	168	52	—	—	—	—	0.22	0.50 – 0.95

TABLE 4.—F_3 BREEDING BEHAVIOUR OF EACH OF THE SEVEN CROSSES COMPARED WITH AN EXPECTED GENOTYPIC 1:2:1 RATIO BY THE X^2 METHOD

Cross	Character	Number of F_3 lines	Observed ratio	Calculated ratio	X^2	P
Chinese × Chinese (2 III Thatcher)	Awning	48	9:28:11	12:24:12	1.50	0.30 – 0.50
Chinese × Chinese (2 IV Thatcher)	Awning	44	10:27: 7	11:22:11	2.68	0.20 – 0.30
Chinese × Chinese (2 XII Thatcher)	Awning	56	17:24:15	14:28:14	1.28	0.50 – 0.95
Chinese × Chinese (2 XXI Thatcher)	Awning	44	7:29:8	11:22:11	3.95	0.10 – 0.20
Chinese × Chinese (2 III Hope)	Pseudo-black chaff	52	10:26:16	13:26:13	1.25	0.50 – 0.95
Chinese × Chinese (2 VII Hope)	Culm colour	76	21:41:14	19:38:19	1.77	0.30 – 0.50
Chinese × Chinese (2 III Timstein)	Awning	60	11:35:14	15:30:15	1.97	0.30 – 0.50

The following hypothesis has been presented elsewhere (3) to explain awn types in lines carrying chromosomes III, IV, XII, and XXI of Thatcher and III of Timstein. It is assumed that either hd* (VIII) or b_2**(X) or both are epistatic to genes on chromosomes III, IV, XII, and XXI whereas Hd or B_2 or both are non-epistatic or only partially epistatic to these same genes. The hypothesis holds whether the genes on these chromosomes are dominant or recessive. With the addition of information on dominance relationships given above the genotypes for Chinese, Thatcher, Timstein, and Chinese substitution lines III, IV, XII and XXI for Thatcher and Timstein chromosomes would be as follows:

	III	IV	VIII	IX	X	XII	XXI
Chinese	A_1A_1	A_2A_2	HdHd	b_1b_1	B_2B_2	A_3A_3	A_4A_4
Thatcher	a_1a_1	a_2a_2	Hd^aHd^a or hdhd	$b_1^ab_1^a$	b_2b_2	a_3a_3	a_4a_4
Timstein	a_1a_1	A_2A_2	hdhd	B_1B_1 or $B_1^aB_1^a$	b_2b_2	A_3A_3	A_4A_4
Chinese (2 III Thatcher) Chinese (2 III Timstein)	a_1a_1	A_2A_2	HdHd	b_1b_1	B_2B_2	A_3A_3	A_4A_4
Chinese (2 IV Thatcher)	A_1A_1	a_2a_2	HdHd	b_1b_1	B_2B_2	A_3A_3	A_4A_4
Chinese (2 XII Thatcher)	A_1A_1	A_2A_2	HdHd	b_1b_1	B_2B_2	a_3a_3	A_4A_4
Chinese (2 XXI Thatcher)	A_1A_1	A_2A_2	HdHd	b_1b_1	B_2B_2	A_3A_3	a_4a_4

The good fit of a 3:1 ratio in the F_2 and F_3 of Chinese × Chinese (2 III Hope) and Chinese × Chinese (2 VII Hope) indicates that Hope chromosomes III and VII each carry only one gene for reaction to pseudo-black chaff and culm colour respectively. If only one gene conditions pigmentation as reported (8, 10) all varieties with purple culms must possess the dominant allele and those with white culms the recessive allele.

The number and dominance relationships of genes on each of the substituted chromosomes and their homologues in the recipient parent are given in Table 5.

TABLE 5.—NUMBER AND DOMINANCE RELATIONSHIPS OF GENES ON CHROMOSOMES III, IV, XII AND XXI

Chromosome	Gene	Character	Source of recessive allele	Source of dominant allele
III	A_1	Awning	Thatcher and Timstein	Chinese
III	Pbc	Pseudo-black chaff	Hope	Chinese
IV	A_2	Awning	Thatcher	Chinese
VII	Pc	Culm colour	Chinese	Hope
XII	A_3	Awning	Thatcher	Chinese
XXI	A_4	Awning	Thatcher	Chinese

* Recessive allele of hooded awn gene (Hd)
** Recessive allele of awn-suppressing gene (B_2)

DISCUSSION

Although characters studied were all found to be monogenically controlled the method should prove equally effective for chromosomes carrying two or more genes, regardless of the type of gene interaction. Except for genes that are ineffective when hemizygous, such as the speltoid-suppressing and non-*sphaerococcum* genes (6) which may be studied only in monosomics and nullisomics, gene effects may be assessed as dominant, incompletely dominant or recessive from F_1's of crosses between substitution lines and recipient varieties. The study of segregation in the F_2 and subsequent generations may be used to establish the number of gene pairs involved.

Aneuploid methods of genetic analysis, such as the study of F_1 monosomics and F_2 and F_3 populations derived from monosomic F_1's, have a threefold purpose. These methods permit (1) the association of genes with specific chromosomes, (2) the determination of the number of genes carried by a specific chromosome and (3) the determination of the effect of the genes. Studies of substitution lines present an over-all effect of transferred whole chromosomes on a particular character and fulfil only the first of the above mentioned functions. The study of substitution lines, therefore, has to be supplemented by an additional study as described in this report to provide a complete genetic analysis of the characters under investigation unless extensive investigations have already been conducted and the number of genes and their effect determined. However, it has the advantage over other methods of permitting the association of minor as well as major genes with chromosomes in addition to its promise as a plant-breeding tool. Furthermore, supplementary studies of the kind described here are essential if linkage associations are eventually to be established among genes for each of the 21 chromosomes in common wheat.

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CHEMICAL COMPOSITION OF ALFALFA AS RELATED TO DEGREE OF TOLERANCE TO MANGANESE AND ALUMINIUM[1]

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ABSTRACT

In an attempt to study the nature of plant tolerance to soil acidity and related factors, a series of investigations was conducted with sand cultures to determine the chemical composition of alfalfa as related to its response to various concentrations of manganese, aluminium and calcium. All clones studied were affected, but to a variable degree, by an excess of manganese and aluminium. They absorbed approximately the same amounts of these elements but considerable differences were noted in their rate of translocation from the roots to the aerial organs. As a result, plants which were the least affected by manganese and aluminium contained smaller amounts of those two elements in their stems and leaves and larger amounts in their roots. Moreover, for a given content of manganese and aluminium in aerial organs, the degree of injury was approximately the same for all clones. More total and water-soluble calcium was found in so-called tolerant than non-tolerant plants. Also, an increase in the calcium concentration of the nutrient culture was effective in reducing manganese toxicity. These results suggest the theory that the rate of uptake of calcium by the plant is one of the factors determining its degree of tolerance to manganese and aluminium.

INTRODUCTION

The failure of legumes to grow properly on highly acid soils is a problem that receives considerable attention from both soil scientists and plant physiologists. Consequently, various theories have been formulated, among which the latest and most strongly advocated has to do with manganese and aluminium toxicity. Some of the initial work conducted at this institution on the tolerance of alfalfa to these elements showed that various clones behaved quite differently in toxic concentrations, indicating that some clones were more tolerant than others to such conditions (9). In an attempt to determine the nature of that tolerance, it was believed advisable to study the chemical composition of alfalfa in relation to its behaviour towards toxic concentrations of manganese and aluminium.

REVIEW OF LITERATURE

One of the first lines of evidence bearing on the subject of soil acidity arose from water-culture experiments, which showed that plant activity was restricted as the pH of the medium fell below 5.0 (3). This type of work produced the hydrogen-ion toxicity theory. Later investigations demonstrated that a substantial increase in the calcium supply would compensate somewhat for an increase in acidity (1, 2). This finding led to the opinion that poor growth of plants on acid soils might be due to calcium deficiency. Recent reports dealing with acid-soil infertility tend to eliminate the calcium-deficiency theory (15, 27, 32). Rather, they agree with earlier workers (6, 7, 8, 11, 12, 13, 18, 21, 22, 24, 28, 29, 31) that toxic concentrations of manganese and aluminium are responsible for the poor growth of plants on acid soils.

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The antagonistic effect of manganese and aluminium on the absorption of calcium by plants has also been demonstrated (10, 33). On the other hand, it has been reported that a liberal supply of calcium in the soil counteracts, to some extent, the toxic action of high concentrations of manganese (5, 17), but not of aluminium (16). Such interrelations between manganese and calcium brought about the theory that the ratio of calcium to manganese in the soil as well as in the plant is just as important a factor as their individual concentrations. Kipps (20) even suggested a ratio of calcium to manganese of 66:1 or greater in alfalfa as being essential to healthy growth. Morris and Pierre (26), however, found no evidence that calcium reduced manganese toxicity.

MATERIALS AND METHODS

Four clonal lines of alfalfa were grown in sand cultures containing variable amounts of manganese, aluminium and calcium. In the complete nutrient solution the major elements were supplied from the four following salts: KH_2PO_4, $(CaNO_3)_{2.4}H_2O$, NH_4NO_3 and $MgSO_{4.7}H_2O$. Manganese, aluminium, zinc and copper were supplied as chloride salts, iron as ferric citrate, boron as boric acid and molybdenum as ammonium molybdate. The resulting concentrations in parts per million of the various ions were as follows: 124 for N, 145 for P, 198 for K, 183 for Ca, 49 for Mg, 65 for S, 0.2 for Zn, 0.1 for Cu, 0.5 for Mn, 15 for Fe, 0.01 for Mo and 0.5 for B. The nutrient solution used was buffered at pH 4.7, in order to avoid oxidation and subsequent precipitation of manganese, iron and aluminium. It is also in soils of pH values lower than 5.0 that manganese toxicity occurs to any appreciable extent.

The chemical composition of alfalfa, as related to its response to various concentrations of manganese, aluminium and calcium, was evaluated in three tests:

Test 1: A study of 4 levels of manganese (0.5, 5, 12.5 and 25 p.p.m.)
Test 2: A study of 4 levels of aluminium (0, 5, 10 and 20 p.p.m.)
Test 3: A study of 4 levels of calcium (0, 50, 100 and 200 p.p.m.) combined with 2 levels of manganese (0.5 and 25 p.p.m.).

The analysis of the nutrient culture at various times during the course of the work indicated that only insignificant amounts of manganese and aluminium became precipitated at the pH of 4.7.

All plants were harvested when the most mature plants reached the early bloom stage of growth. At the time of harvesting, notes of the presence and severity of nutritional symptoms were taken and the yields of green matter recorded. For the determination of total manganese, aluminium, calcium and phosphorus in the plant, a 2-gram sample of the ground tissue, previously dried for 72 hours at 70° C., was subjected to dry ashing at 450° C. for 12 hours. The ash was then taken up in 6N hydrochloric acid and aliquots of the resulting solution analysed. For the determination of the so-called "active" calcium and manganese in the aerial organs of the plant, a 2-gram sample of the freshly harvested material was triturated in 25 ml. of distilled water. Fresh root material, being quite lignified, could not be treated in the same manner. In order to avoid oxidation due to

slow drying, freshly collected roots were dehydrated with dry ice. One gram of the ground material was agitated in 25 ml. of distilled water for 5 minutes.

Manganese was determined colorimetrically, following oxidation to permanganate with potassium periodate (25). The method proposed by Peech and English (30), using the ammonium salt of aurin-tricarboxylic acid (aluminon) was chosen for the determination of aluminium. Calcium was measured turbidimetrically according to the method proposed by the same authors (30). The ammonium molybdate method was used for determining phosphorus (19).

In all tests, green matter yields and nutritional symptoms were taken as indicators of the degree of tolerance of a plant to toxic levels of manganese and aluminium. Six cuttings were made from each test. Only the results from the third and sixth cuttings are reported as they represent the changes which occurred during the course of the tests. Due to the extreme difficulty encountered in washing sand away from the smaller roots, only the larger ones were ground for chemical analysis.

RESULTS AND DISCUSSION

Manganese and Aluminium Toxicity Symptoms

Symptoms of manganese toxicity on alfalfa can be described as follows: Small chlorotic spots first appear near the margin of the lower leaves, and as the severity increases, spots also develop on the middle and upper leaves. Later, a downward cupping of most of the leaves having a chlorotic margin is observed. In extreme cases, a sort of crinkling, which gives the leaf a raggy appearance, develops. Finally, the entire plant wilts and dies.

TABLE 1.—YIELD, SYMPTOMS OF MANGANESE TOXICITY, AND MANGANESE CONTENT OF FOUR CLONAL LINES OF ALFALFA GROWN IN SAND CULTURES CONTAINING VARYING AMOUNTS OF MANGANESE

Clonal line	Soluble Mn in sand culture, p.p.m.	Yield: Green weight gm./jar		Symptoms at		Mn in plant (p.p.m. oven-dry basis)		
						Tops		Roots
		3rd cut	6th cut	3rd cut	6th cut	3rd cut	6th cut	6th cut
49–2700	0.5	38	63	None	None	143	150	200
	5.0	20	39	Light	Light	268	241	297
	12.5	8	15	Medium	Severe	399	417	449
	25.0	5	3	Severe	Severe	443	479	443
49–3901	0.5	37	65	None	None	167	138	174
	5.0	35	52	Very light	Light	235	250	390
	12.5	24	25	Light	Light	290	305	409
	25.0	15	14	Medium	Severe	326	403	506
49–4361	0.5	50	62	None	None	160	181	208
	5.0	33	58	Very light	Very light	249	222	290
	12.5	32	52	Light	Light	303	319	412
	25.0	24	40	Light	Light	305	321	560
49–5885	0.5	54	44	None	None	159	132	181
	5.0	45	49	None	None	201	175	341
	12.5	35	40	Very light	Light	266	284	500
	25.0	36	34	Light	Light	295	326	625
L.S.D. (5%)		6.6	9.4					

The only symptoms brought about by an excess of aluminium on alfalfa, within the concentrations used in these experiments, (from 0 to 20 parts per million in the sand cultures) consisted of chlorotic spots on the leaves, particularly the lower ones.

The above symptoms have been developed in the greenhouse under conditions of manganese toxicity which varied from severe to extreme. Under field conditions, however, the only abnormalities observed in Eastern Quebec are the chlorosis and downward cupping of the leaves. Unfortunately, some other factors limiting plant growth, such as aluminium toxicity, calcium deficiency, fixation of phosphorus, etc., are generally associated with manganese toxicity in very acid soils, making the diagnosis of manganese toxicity symptoms very difficult.

Effects of Manganese Toxicity

As shown in Table 1, the results of the first test indicate that the four clonal lines studied had approximately the same productivity index, when grown in sand cultures containing normal concentration of manganese, i.e., 0.5 part per million. However, they behaved quite differently, when grown in toxic concentrations. In fact, clone 49-2700 was almost dead at the sixth cutting, whereas clones 49-4361 and 49-5885 were only slightly affected. Clone 49-3901 was moderately affected, but to a lesser degree than clone 49-2700. The four clonal lines studied showed a wide range of tolerance to manganese toxicity.

The symptoms of manganese toxicity were of similar intensity at both the third and sixth cuttings. Similarly, chemical analysis showed only slight differences in the manganese content of the aerial portion of the plants for these two cuttings. Increasing the amount of soluble manganese in the substrate resulted in higher contents in all clones, including those having a greater degree of tolerance to toxic conditions.

The total amount of manganese in the entire plant, roots and aerial portion, at the sixth cutting, was approximately the same for all clones. Large differences, however, were observed between clones, as to the distribution of manganese within the plant. The more tolerant clones contained less manganese in their stems and leaves and more in their roots than those which were severely injured. Thus, the differential translocation of manganese from roots to aerial organs seems to account for the different degrees of tolerance noted in the clones studied. This is further substantiated in that a given class of toxicity symptoms corresponds to rather constant concentrations of manganese in the aerial portion of all clones. This means that tolerant and non-tolerant plants are likely to exhibit approximately the same symptoms and be injured to about the same degree, if they contain approximately the same amounts of manganese in their stems and leaves. The following scale, gives the concentration of manganese in the aerial organs of the plant as related to the nutritional disturbances observed:

Severity of manganese toxicity	*Manganese in stems and leaves* (p.p.m.)
None	Less than 175
Very light (chlorosis)	175 to 250
Light (chlorosis and cupping)	250 to 325
Medium (crinkling of some leaves)	325 to 400
Severe (crinkling of most leaves)	Over 400

TABLE 2.—YIELD, SYMPTOMS OF ALUMINIUM TOXICITY, AND ALUMINIUM AND PHOSPHORUS CONTENT OF FOUR CLONAL LINES OF ALFALFA GROWN IN SAND CULTURES CONTAINING VARYING AMOUNTS OF ALUMINIUM

Clonal line	Soluble Al in sand culture, p.p.m.	Yield: Green wt. gm./jar		Symptoms at		Al in plant (p.p.m. oven-dry basis)			P in plant % oven-dry basis
						Tops		Roots	
		3rd cut	6th cut	3rd cut	6th cut	3rd cut	6th cut	6th cut	6th cut
49–2700	0	54	74	None	None	41	58	43	.48
	5.0	47	52	None	Light	207	281	397	.37
	10.0	28	25	Light	Severe	326	464	604	.25
	20.0	15	16	Medium	Severe	417	559	822	.29
49–3901	0	47	73	None	None	37	23	22	.50
	5.0	35	52	None	Light	178	259	407	.50
	10.0	30	41	Medium	Medium	320	391	837	.29
	20.0	37	29	Medium	Severe	437	442	1025	.33
49–4361	0	58	78	None	None	65	53	71	.56
	5.0	46	61	None	None	162	186	368	.45
	10.0	49	55	None	Light	233	303	790	.44
	20.0	47	59	None	Light	229	318	1079	.38
49–5885	0	55	78	None	None	49	78	55	.45
	5.0	51	63	None	None	154	162	506	.35
	10.0	49	52	None	Light	141	276	918	.33
	20.0	35	43	Light	Light	307	325	1150	.32
L.S.D. (5%)		7.3	5.1						

Effects of Aluminium Toxicity

Results from the second test show that the four clonal lines studied behaved in the presence of aluminium toxicity in just about the same manner as they had in the presence of manganese toxicity (Table 2). However, aluminium, within the concentrations used, (from 0 to 20 parts per million) was not as toxic to alfalfa as manganese had been.

Increasing the amount of soluble aluminium in the sand cultures from 0 to 20 parts per million resulted in reduced yields, especially in the case of clones 49-2700 and 49-3901. With increasing aluminium in the substrate its concentration in the plants was increased by as much as tenfold in the case of leaves and stems, and twentyfold in the case of roots, whereas the concentration of phosphorus in the aerial organs was concurrently reduced by only one-third. Moveover, the depressive action of toxic concentrations of aluminium on phosphorus uptake was of approximately the same order for all clones. Therefore, interaction between aluminium and phosphorus does not seem to be responsible for the variable degree of tolerance observed. The translocation of aluminium from the roots to the leaves and stems, like that of manganese, was greater in the case of clones 49-2700 and 49-3901, i.e., those which were the most affected by toxic concentrations of aluminium. This seems to indicate that the aluminium translocated from the roots to the aerial organs, rather than that taken up by the plant, was responsible for the differential responses noted.

All plants, regardless of their tolerance, exhibited approximately the same symptoms if they happened to contain about equal amounts of aluminium. The following scale shows the relation between the symptoms due to aluminium toxicity on alfalfa and the amounts of that element contained in the aerial organs of the plant:

Severity of aluminium toxicity	*Aluminium in stems and leaves* (p.p.m.)
None	Less than 200
Light	200 to 325
Medium	325 to 450
Severe	Over 450

TABLE 3.—YIELD AND NUTRITIONAL SYMPTOMS OF FOUR CLONAL LINES OF ALFALFA GROWN IN SAND CULTURES CONTAINING VARYING AMOUNTS OF CALCIUM ASSOCIATED WITH NORMAL AND TOXIC CONCENTRATIONS OF MANGANESE

Clonal line	Ca in sand culture p.p.m.	Yields and foliage symptoms			
		Green wt.—gm. per jar average of 6 cuttings		Symptoms at 6th cut	
		0.5 p.p.m. Mn	25 p.p.m. Mn	0.5 p.p.m. Mn	25 p.p.m. Mn
49–2700	0	7*	2*	Ca deficiency**	Dead since 2nd cut
	50	77	8	None	Dead since 3rd cut
	100	90	24	None	Severe Mn toxicity
	200	84	29	None	Severe Mn toxicity
49–3901	0	8	3	Ca deficiency	Dead since 3rd cut
	50	62	7	None	Dead since 3rd cut
	100	85	17	None	Severe Mn toxicity
	200	98	23	None	Medium Mn toxicity
49–4361	0	10	2	Ca deficiency	Dead since 4th cut
	50	76	19	None	Severe Mn toxicity
	100	90	35	None	Severe Mn toxicity
	200	93	43	None	Medium Mn toxicity
49–5885	0	8	3	Ca deficiency	Dead since 2nd cut
	50	70	25	None	Severe Mn toxicity
	100	82	40	None	Medium Mn toxicity
	200	96	47	None	Medium Mn toxicity
L.S.D. (5%)		12.7	8.2		

* Under the conditions of the experiment, it was impossible to obtain a substrate completely free from calcium.

** Chlorotic spots due to manganese toxicity were present on the first two harvests, i.e. before the effects of calcium deficiency became too severe.

Relation between Calcium Supply and Manganese Toxicity

Raising the calcium level of the nutrient culture, as shown in Table 3, resulted in considerably increased yields with either normal or toxic concentrations of manganese. With normal supply of manganese (0.5 p.p.m.), all clones studied gave approximately the same yields, for any given level of calcium in the sand culture. This substantiates a previous statement that these clones had approximately the same productivity index under normal conditions. With toxic level of manganese (25 p.p.m.), however, and sufficient calcium, clones 49-4361 and 49-5885 gave higher yields and exhibited milder symptoms of manganese toxicity. These two clones were equally the most tolerant to manganese toxicity in the previous tests. Calcium deficiency affected all four clones to almost the same extent. Increasing the calcium level of the substrate resulted in decreased severity of manganese toxicity symptoms. Even the normal level of manganese, without calcium, induced manganese toxicity in the early life of the plant, i.e., before the calcium deficiency became severe enough to mask other symptoms.

Plant analytical data for total and so-called "active" (water-soluble) calcium and manganese are summarized in Table 4 for the normal level of manganese and in Table 5 for the toxic level. At either level of manganese, all parts of clones 49-4361 and 49-5885 contained appreciably more total and active calcium than the other two, except when grown in cultures free of calcium. They also contained less "active" manganese in their roots and total manganese in their aerial organs. The total manganese content of the roots remained rather constant for all clones when grown with normal

TABLE 4.—CHEMICAL COMPOSITION OF FOUR CLONAL LINES OF ALFALFA GROWN IN SAND CULTURES CONTAINING VARYING AMOUNTS OF CALCIUM ASSOCIATED WITH A NORMAL CONCENTRATION (0.5 p.p.m.) OF MANGANESE

Clonal line	Ca in sand culture, p.p.m.	Ca (av. of 6 cuttings): per cent				Mn (av. of 6 cuttings): p.p.m.			
		Total (oven-dry basis)		H_2O-Soluble (green wt. basis)		Total (oven-dry basis)		H_2O-Soluble (green wt. basis)	
		Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
49–2700	0	0.51	0.36	.02	.04	203	207	11	96
	50	1.17	1.04	.06	.23	130	236	14	83
	100	2.21	2.10	.10	.41	137	199	10	77
	200	2.75	2.49	.15	.56	119	231	6	59
49–3901	0	0.48	0.41	.03	.01	180	244	10	92
	50	1.29	1.22	.05	.20	123	215	10	68
	100	2.34	2.29	.12	.35	122	261	14	72
	200	2.96	2.35	.15	.61	105	206	9	60
49–4361	0	0.64	0.38	.05	.07	174	182	16	84
	50	1.44	1.75	.09	.33	102	225	9	66
	100	2.43	2.50	.14	.49	95	239	12	45
	200	3.10	2.74	.18	.73	79	201	11	49
49–5885	0	0.58	0.54	.02	.11	135	253	12	85
	50	1.35	1.41	.07	.30	111	192	17	70
	100	2.50	2.79	.12	.5	108	214	15	51
	200	3.01	2.83	.16	.6	87	229	5	54

TABLE 5.—CHEMICAL COMPOSITION OF FOUR CLONAL LINES OF ALFALFA GROWN IN SAND CULTURES CONTAINING VARYING AMOUNTS OF CALCIUM ASSOCIATED WITH A TOXIC CONCENTRATION (25 p.p.m.) OF MANGANESE

Clonal line	Ca in sand culture p.p.m.	Ca (av. of 6 cuttings): per cent				Mn (av. of 6 cuttings): p.p.m.			
		Total (oven-dry basis)		H_2O-Soluble (green wt. basis)		Total (oven-dry basis)		H_2O-Soluble (green wt. basis)	
		Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
49–2700	0	0.40	0.50	.01	.01	561	407	31	130
	50	0.94	0.77	.04	.12	475	440	17	93
	100	2.24	1.45	.06	.23	458	476	12	85
	200	2.42	1.92	.05	.41	404	473	14	71
49–3901	0	0.38	0.39	.01	.01	497	503	39	114
	50	1.20	0.66	.05	.09	446	472	20	85
	100	2.18	1.27	.10	.35	396	496	19	73
	200	2.22	2.04	.08	.37	368	461	15	87
49–4361	0	0.59	0.51	.02	.02	406	549	25	91
	50	1.31	1.03	.06	.25	436	477	16	68
	100	2.22	1.74	.09	.38	390	501	16	65
	200	2.84	2.31	.12	.59	307	514	11	50
49–5885	0	0.47	0.45	.01	.02	492	562	29	82
	50	1.40	0.86	.05	.19	414	503	23	56
	100	2.30	1.80	.10	.35	347	497	13	63
	200	2.97	2.26	.10	.53	301	531	10	38

supply of manganese (Table 4), whereas it had a tendency to be higher for the tolerant than non-tolerant clones when grown in substrate containing toxic levels of manganese (Table 5). The uptake of calcium was reduced appreciably by increasing the concentration of manganese of the culture solution from 0.5 to 25 parts per million. Therefore, a positive relationship was observed between the degree of tolerance of alfalfa to toxic levels of manganese and their calcium content.

It is concluded that the ability of resistant plants to keep part of their absorbed calcium in "active" form is an important factor determining their

degree of tolerance to manganese and presumably aluminium toxicity This follows from the observations that increase in the calcium concentration of the nutrient culture counteracts, to some extent, the action of manganese, and the plants which grow best in toxic concentrations of manganese contain more calcium. Whether this is due to the development of the root system or some plant nutrition phenomenon, such as variable exchange capacity. or the plant metabolism as a whole is not known. A few observations were made in the present tests on root systems but the results obtained are not conclusive. Further work needs to be done on the subject. Smith (34) and Lyness (23) found differences in phosphorus utilization between strains and hybrids of corn and attributed this to a greater proportion of secondary to primary roots on efficient inbred lines and hybrids. Harvey (14) suggested that the differential nutritional responses which he observed between corn inbreds were due to inherent differences in the genetic constitution of the strains.

Calcium in the plant seems to favour the precipitation and consequent immobilization of excess manganese within the roots. Consequently, a greater content of calcium, especially "active" calcium, in the roots would prevent the translocation of excess manganese to the aerial organs. Furthermore, this would indicate that an excess of manganese becomes detrimental only when enough of the element has moved from the roots to the aerial organs. Therefore, the determination of manganese in the stems and leaves should give a good indication of the extent of that toxicity. Whether this assumption applies to alfalfa only or to other plant species also is not known.

Briefly, it may be said that the degree of tolerance of alfalfa to toxic concentrations of manganese and aluminium appears to be related to the plant efficiency in absorbing calcium from the substrate and the inherent ability of the roots in maintaining some of that absorbed calcium in an active form. Calcium is believed to act in two ways: first, it lowers the uptake of manganese and aluminium by the roots, and, second, it immobilizes part of the absorbed manganese and aluminium within the roots, with the results that the quantities translocated from the roots to the aerial organs are appreciably reduced.

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THE INHERITANCE OF RESISTANCE TO *ASCOCHYTA PISI* LIB. IN PEAS[1]

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ABSTRACT

As part of a program for breeding horticultural varieties of peas resistant to *Ascochyta pisi* Lib., a study was made of the inheritance of resistance to a monospore isolate of the pathogen using a cross between the resistant pea strain, Ottawa A-100, and the susceptible variety, Thomas Laxton. The results indicate that resistance to the isolate used is due to duplicate dominant genes, either one of which will give resistance.

INTRODUCTION

For a number of years the seed-transmitted disease, leaf and pod spot caused by *Ascochyta pisi* Lib., has been a serious economic factor in the pea-growing areas of Canada. Disease surveys (5) during 1939-50 have shown that in Canada *A. pisi* is more prevalent on pea seed than is either of the related organisms *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr. or *Ascochyta pinodella* L. K. Jones. This may be partially explained by the fact that the disease caused by *M. pinodes* may often kill pea plants before they mature, whereas the disease caused by *A. pisi* does not usually kill the infected plants but may attack the pods in such a way as to infect the seed. Because of its infection locus the foot rot organism, *A. pinodella*, does not tend to be seed-borne to the same degree as *A. pisi*. In areas of concentrated production either for seed or for processing *A. pisi* can cause widespread damage when climatic conditions are favourable for its growth.

It has long been known that there are varietal differences in reaction to *A. pisi*. In 1927 L. K. Jones (3) found partial resistance in several varieties of peas, but this resistance was not sufficient to be of much practical importance. In inoculation tests at Ottawa (4) in 1939 a selection from the cross V.C.×American Wonder was found to be resistant to 18 out of 20 isolates of *A. pisi* from different sources. A line designated A-100 was eventually selected from this cross and proved highly resistant to several isolates of the fungus. In addition Gilpatrick and Busch (2) found that A-100 was resistant to several isolates from different sources. Brewer and MacNeill (1) in a study of the nature of resistance to *A. pisi* found A-100 to be very highly resistant, and considered that resistance must be due to some physiologic factor in the host which tends to limit colonization to the incipient stage of development. Wallen (6) has found that in Canada there are at least four physiologic races, which he designated I to IV, and that with minor exceptions each race appears to have a geographically limited distribution. Wark (7) in Australia found that resistance, carried by the field pea, Austrian Winter, was dominant to susceptibility and was due to

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"three mendelian factors, all of which must be present for the manifestation of resistance". These conclusions differ from preliminary results obtained at Ottawa* in 1952. In the latter test, crosses between A-100 and two susceptible varieties, Alton and Thomas Laxton, were studied for their reaction to two different isolates of *A. pisi*. None of these varieties was included in Wark's studies. Results of the Ottawa test indicated that resistance might be due to three dominant factors, any one of which could give resistance. These conclusions were based on bulked data from the progenies of two different crosses, using populations which may have been too small. The present studies were carried out with the intention of either confirming or disproving the 1952 results.

MATERIALS AND METHODS

The parent varieties, the F_1 and the F_2 progenies of the cross Thomas Laxton×A-100, were studied for their reaction to inoculation with a culture of race II of *A. pisi* isolated in 1949 from diseased peas found near Ottawa. The study was first carried out in 1954 and repeated in 1956. Since the seed supply did not permit the immediate repetition of this test, it was necessary to repeat the cross and grow F_1 and F_2 progenies from it for the 1956 test.

Pea seeds were sown in shallow 5-inch pots at the rate of 10 seeds per pot, and grown in a greenhouse at 18° C. for 14 days after seeding. At the end of the 14-day period the leaves and stems were lightly abraded with 2/0 sandpaper. They were then sprayed with a pycnospore suspension derived from a monospore isolate of race II of *A. pisi* at the rate of approximately 5 ml. per pot, using a No. 287 de Vilbiss atomizer. After inoculation the plants were held in a constant temperature incubator at a high relative humidity for at least 8 hours at 18° C. They were then removed and grown in a greenhouse under approximately the same conditions of temperature and humidity. After 4 days in the greenhouse the plants were examined for symptoms of infection.

Since in certain varieties it has been found that stems and leaves do not necessarily give the same reactions to infection, the following system of rating was used:

Degree of resistance	Reaction designation		Description of reaction
	Leaves	Stems	
Highly resistant	A	1	Small flecks only
Moderately resistant	B	2	Small lesions, no pycnidia
Moderately susceptible	C	3	Necrotic lesions, pycnidia present
Very susceptible	D	4	Deep necrotic lesions, abundant pycnidia
Highly susceptible	E	5	Plants killed

With this rating a plant might be classed as A-1, B-2, A-3, etc., depending on the severity of stem and leaf infection. In differentiating between resistance and susceptibility, all plants rating more than B for leaf infection or 2 for stem infection were classed as susceptible.

*Lyall, L. H. Pea breeding for resistance to *Ascochyta pisi* Lib. I. Reaction of pea varieties, selections, and crosses to artificial inoculation. Unpublished paper delivered at Annual Meeting of the Agricultural Institute of Canada, 1952.

EXPERIMENTAL RESULTS

The segregation of resistant and susceptible classes in the F_2 generation was clear-cut in both the 1954 and 1956 studies. Thomas Laxton was highly susceptible while A-100, and the F_1 of Thomas Laxton×A-100 were highly resistant.

The data on the classification of this material in both tests are presented in Table 1.

TABLE 1.—REACTION OF PARENT VARIETIES, AND F_1 AND F_2 PROGENIES OF THE CROSS THOMAS LAXTON×A-100 TO INOCULATION WITH *A. pisi*, RACE II

Variety or progeny	Number of plants			P for 15:1 ratio
	Resistant	Susceptible	Total	
1954				
Thomas Laxton	0	45	45	
A-100	48	0	48	
Thomas Laxton×A-100 F_1	46	0	46	0.95–0.99
Thomas Laxton×A-100 F_2 Observed	349	23	372	
Expected	348.74	23.26		
1956				
Thomas Laxton	0	49	49	
A-100	41	0	41	
Thomas Laxton×A-100 F_1	50	0	50	
Thomas Laxton×A-100 F_2 Observed	355	24	379	0.95–0.99
Expected	355.31	23.69		

In both years the parent varieties behaved as expected, the F_1 populations were all resistant, and a satisfactory fit to a 15:1 ratio was obtained in both segregating populations.

DISCUSSION OF RESULTS

These and previous tests show that the variety A-100 carries a high degree of resistance to race II of *A. pisi*, and that this resistance is dominant. Results of a preliminary study in 1952 indicated the possibility that resistance might be due to three dominant factors, any one of which could give resistance. However, in the present studies the F_2 segregation ratio of 15 resistant to 1 susceptible indicates that resistance is due to duplicate dominant factors, either one of which will give resistance.

The results presented here differ from those of Wark (7) who obtained a ratio of 27 resistant to 37 susceptible in the F_2 and concluded that three factors were involved, all of which were necessary in the dominant condition to produce resistance. However, his conclusions were based on bulked data from crosses involving a number of different pea varieties and may possibly have involved more than one race of the fungus. The present study deals with the reactions of segregating progenies from a cross between two varieties, to inoculation with a single race of the pathogen. Neither of these varieties was used by Wark and it is quite possible, therefore, that different genes for resistance are involved in the two studies.

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INHERITANCE OF EARLINESS IN BARLEY[1]

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ABSTRACT

The F_1, F_2 and F_3 of seven crosses between spring barley varieties of differential maturity periods were grown in the same year. Genetic analyses were largely based on F_3 breeding types, six of which were distinguishable among frequency distributions of F_3 hybrids classified in days-to-heading categories. Each breeding type was determined by characteristic variance and mean days-to-heading values. It was hypothesized that parents in each late × early cross differed by additive, increaser alleles at two loci, giving $a_1a_1b_1b_1 \times a\,a\,b\,b$. Theoretically, such a cross produces six F_2 breeding types, viz., late, intermediate, and early homozygotes, and late, intermediate, and early heterozygotes; the ratio being 1:2:1:4:4:4. Observed frequencies being in good agreement with these expectations, it was concluded that the hypothesis accounted satisfactorily for the main features of inheritance. Minor discrepancies were attributed to modifying genes, the nature of which could not be determined.

INTRODUCTION

The phenotypic expression of a quantitative character such as earliness may be considered as the resultant of two interacting factors, the genotype and the environment.

Although environmental effect is studied herein only as a factor to be controlled, or eliminated, it is important to examine the many ways in which the environment affects the earliness characteristic in barley; Bell (1) stressed the relationship between physiology, heredity and environment in the expression of earliness. The time at which a particular cereal variety comes into head is the manifestation of a complex series of physiological processes within the plant. He found the light requirement to be an exceedingly important factor in determining earliness in barley; for example, a plant which is early in one area may react quite differently in another area where the light intensity and duration differed. The importance of light duration is further indicated by the study of Johnson and Taylor (3) on the effects of photoperiod and temperature on the development of spike primordia in barley. Other workers, as reported by Smith (7), found that temperature, nitrogen content of the soil, sand content of the soil, and special concentrations of auxins and coumarin were significant influences on time of maturity in barley.

On the genetical side, workers, as reviewed by Smith (7), have found the expression of earliness to be dependent on from one to many genes. Griffee (2) reports a single gene pair responsible for earliness and that the gene for earliness is dominant. Johnston (4), and Johnston and Aamodt (5) studied the F_3 generation from a cross between an early and a late variety and considered that earliness was best explained on the basis of

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polymeric genes. They found that time of heading is so influenced by environmental factors that data pertaining to individual plants are of no great significance, and also that it is impractical to use parental distributions as a measure of homozygosity. Their conclusions were that earliness was dependent on two or more gene pairs and that transgressive segregation for both earliness and lateness was clear. Wilson (8) conducted one of the first genetic studies of early maturity and indicated that earliness was recessive to lateness. Bell (1) postulated that, within a pure line, normally developed plants will occupy a period of from 4 to 5 days to accomplish over-all ear emergence in a plot of 200 to 250 plants. He found that seeding F_1, F_2 and F_3 generations in successive years was much less valuable than seeding all generations under study within the same year and at the same date. The F_1 generation in his study inclined toward the later parent and the F_2 reacted oppositely. Transgressive segregation appeared in some crosses.

The object of the present study is to determine, as accurately as possible, the inheritance of earliness in barley in a series of crosses with a view, first, to contributing to genetic knowledge of the character and, second, to giving practical genetic guidance in breeding programs designed to produce superior, early barley varieties.

MATERIALS AND METHODS

The study was based on seven crosses between varieties of spring barley (*Hordeum vulgare L.*) differing in maturity periods as follows:

early × early—
Atsel × Tulare

late × late—
Frontier × Bonneville

early × late—
Atsel × Bonneville
Atsel × Frontier

late × early—
Montcalm × Atsel
Montcalm × Beecher
Montcalm × Tulare.

The F_2 generations of all crosses were grown in the field in 1950. Each F_2 was divided into three parts or "replications" (A, B, and C) which were sown at 15-day intervals. In 1951, the F_2 of all crosses were again sown, together with the F_1 of three crosses. The F_3 was also sown in 1951, maintaining the distinction of the "replicates" but sowing all on the same day.

The summer of 1951, during which the materials under analysis were grown, was highly favourable. At no time was there a lack of moisture, nor did the temperature change appreciably during the vegetative and heading periods. The land used was level, uniform and approximately square in shape. Provision for detecting non-heritable variation was made by growing parental rows between every five F_3 lines.

The F_3 generations, each associated with a particular F_2 phenotype (days-to-heading), were sown in individual 13-foot rows, 1 foot apart. After emergence, plants were thinned to a uniform spacing of three inches in the row. The F_1 and F_2 were sown 3 weeks later than the F_3.

Heading notes were taken each day from the time the first plant headed until the heading period was completed. In all, date of heading was recorded for about 45,000 plants. The criterion used to classify a plant as being headed was the extrusion of the top floret above the flag leaf. Days to heading was taken to be the measure of the earliness of a plant. Previous workers (1, 4, 5) have found date of ear extrusion to be a more reliable measure of earliness than the actual date of maturity.

Arithmetic means and variances were calculated for each row of material, including parental varieties.

The mean days to heading of an F_3 line, $\bar{D}$, is much less subject to environmental influences than the days to heading of a single F_2 plant. Therefore, the $\bar{D}$ of each F_3 line is used to identify the phenotype of the F_2 parental plant from which it was derived.

The variance, V, is used as a measure of the variability within each row. In parental rows V consists of a component due to non-heritable sources only, since parental varieties are considered to be pure lines. Each of the F_1 plants resulting from a cross between two pure lines has the same genotype; therefore, the V of the F_1 consists of a non-heritable component only. In the F_2, segregation of genes occurs, and the F_2 distributions contain an heritable component of variation in addition to the non-heritable portion common to non-segregating lines. The increased V of the F_2 over parents and F_1 is a function of the number of gene differences between the two parents (neglecting the effects of linked genes, epistasis and dominance).

Some of the genotypes in the F_2 will be like the parental genotypes, and the proportion of parental recoveries among F_2 segregates is an indication of the number of differential loci involved. Thus, each parent recovered once in 4 denotes a monohybrid, once in 16 a dihybrid, etc.

The test of the genotype is in its progeny. If a parental genotype occurs among the F_2 segregates it will give an F_3 line phenotypically like the parent and with equal V. The ratio of segregating to non-segregating F_3 lines is an indication of the number of genes in which the parent varieties differ. An average V of the parental varieties was calculated to assess the separate V values of the F_3 lines and thereby arrive at an estimate of the number of homozygous lines in the F_3 generation.

In any monohybrid cross, the theoretical ratio of segregating to non-segregating lines is 1:1. The phenotypes are divided into 3 classes, 1 AA : 2 Aa : 1 aa, the non-segregating lines being distributed in the extreme classes. In a dihybrid cross the phenotypic classes are as follows:

Ratio	1	:	4	:	6	:	4	:	1
Genotypes	(1) AABB		(2) AaBB (2) AABb		(4) AaBb (1) AAbb (1) aaBB.		(2) Aabb (2) aaBb		(1) aabb

Here the ratio of segregating to non-segregating F_3 lines is 3:1. The 4 (out of 16) non-segregating F_3 lines are distributed in the first, third and fifth classes in a ratio of 1:2:1. Similarly, it may be shown in the trihybrid

that the ratio of segregating to non-segregating F_3 lines is 7:1. This is the theoretical basis for the genetic analysis of Table 2.

Assuming additive effects (no dominance) of alleles, a monohybrid will give F_3 lines with the following distributions:

1 a_ia_i parent A
2 a_ia F_1
1 aa parent B.

If a_i denotes an allele increasing the time to heading, parent A becomes the late and parent B the early parent.

On the same assumption the dihybrid cross, $a_ia_ib_ib_i$ (late) × aabb (early), will give:

1 $a_ia_ib_ib_i$ 1 homozygous late

2 $a_ia\ b_ib_i$ } 4 heterozygous moderately late
2 $a_ia_ib_ib$ }

4 $a_ia\ b_ib$ 4 heterozygous intermediate

1 $a_ia_i b\ b$ } 2 homozygous intermediate
1 a a b_ib_i }

2 $a_ia\ b\ b$ } 4 heterozygous moderately early
2 a a b_ib }

1 a a b b 1 homozygous early.

The homozygous-late F_3 line will approximate the late parent for both variance (V) and mean date to head ($\bar{D}$). The heterozygous-late lines will be approximately intermediate between the homozygous late and the heterozygous intermediate for both V and $\bar{D}$. The heterozygous-intermediate lines will approximate the F_2 for both V and $\bar{D}$. The homozygous-intermediate lines will approximate the parental average for V and the F_2 for $\bar{D}$. The heterozygous-early lines will be approximately intermediate between the homozygous early and the heterozygous intermediate for both V and $\bar{D}$. The homozygous-early lines will approximate the early parent for both V and $\bar{D}$. This is the theoretical basis for the main genetical analysis given in Tables 3 to 7 inclusive.

EXPERIMENTAL RESULTS

The F_1 and F_2 Generations

In 1951, F_1 generations (three crosses) and F_2 generations (all seven crosses) were grown in the field.

Table 1 gives the mean days to heading ($\bar{D}$) and variance (V) for parental, F_1 and F_2 as calculated from frequency distributions of plants in days-to-heading classes. The F_2 data were taken from the replicate having a seeding date comparable to that of the F_1.

Where comparisons may be made between parents, F_1 and F_2, it will be seen that parental and F_1 variances are quite similar indicating the presence of a non-heritable component only, while the F_2 variances are relatively much larger, indicating the presence of both non-heritable component (common to all variances) and a very substantial heritable component reflecting F_2 segregation.

TABLE 1.—COMPARISONS OF MEAN DAYS TO HEADING (D) AND VARIANCE (V) OF PARENTS, F_1 AND F_2

Cross	Class	Parent A		Parent B		F_1		F_2		
		$\bar{D}$	V	$\bar{D}$	V	$\bar{D}$	V	$\bar{D}$	V	No.
Atsel × Tulare	Early × early	42.7	0.82	43.7	0.88	46.3	1.04	48.3	9.87	225
Atsel × Bonneville	Early × late	43.4	1.41	60.7	3.70	52.5	1.45	53.4	13.67	138
Atsel × Frontier	Early × late	43.8	2.43	58.5	3.25	—	—	54.7	12.64	274
Montcalm × Atsel	Late × early	54.7	1.33	43.4	0.86	—	—	51.8	19.27	207
Montcalm × Beecher	Late × early	54.1	0.91	45.3	3.86	—	—	46.8	17.45	146
Montcalm × Tulare	Late × early	54.1	0.91	44.1	0.94	—	—	46.9	28.24	13
Frontier × Bonneville	Late × late	57.9	1.45	60.0	1.54	55.3	1.30	60.9	14.15	57

Comparing $\bar{D}$ values in the early × early cross it will be seen that the F_1 is about 3 days and the F_2 5 days later than the parents. This might represent partial dominance of lateness with transgressive segregation for lateness. On the other hand, in the late × late cross the F_1 is some three of four days earlier than the parents while the F_2 is somewhat later than the parental average. This would appear to suggest partial dominance of earliness. Taken together, these observations are considered not to contradict the hypothesis of no dominance (additive alleles).

In the crosses between early varieties and the late varieties, Frontier and Montcalm, $\bar{D}$ values for the F_2 tend to be more or less intermediate between those of the parents. Inspection of the actual distributions shows tendencies for transgressive segregation, which is toward lateness in Atsel × Frontier and Montcalm × Atsel and toward earliness in Montcalm × Beecher and Montcalm × Tulare. This is particularly notable in Montcalm × Beecher, which suggests partial dominance of earliness.

In the early × late cross, Atsel × Bonneville, the $\bar{D}$ value for the F_1 is almost exactly intermediate between those of the parents and corresponds closely to that of the F_2. This is the situation expected where inheritance is governed by cumulative alleles with no expression of dominance. Substituting our $\bar{D}$ values in Mather's (6) formula for additive genes, $4\bar{F}_2 = 2\bar{F}_1 + \bar{P}_1 + \bar{P}_2$, we have:

$$4(53.4) \simeq 2(52.5) + 43.4 + 60.7$$
$$\text{and, } 213.6 \simeq 105.0 + 43.4 + 60.7$$
$$\text{and, } 213.6 \simeq 209.1.$$

Since the values in the equation are approximately equal, we may consider that additive (cumulative) genes determine inheritance in the cross. Mather's mid-parent ($\bar{P}$) test for non-dominance in the F_1 is also reassuring:

$$\frac{\bar{P}}{\frac{43.4 + 60.7}{2}} = \bar{F}_1 \simeq 52.5$$
$$\text{and, } 52.1 \simeq 52.5.$$

These observations on the F_1 and F_2 and parental distributions have suggested different inheritance phenomena in different crosses: partial dominance of earliness, and of lateness; transgressive segregation for earliness, and for lateness; and have strongly suggested additive genic effect in one case. These differences are to be expected in studies of

quantitative inheritance in which environmental factors may fluctuate in degree and direction of influence. Specifically, some variation, which has been differential as between crosses, was probably introduced by the different dates of sowing in the F_2.

These environmental factors came under better statistical control in the study of F_3 lines, where the average of many plants, rather than a single plant, was used to distinguish individual F_2 genotypes.

Correlation Between F_2 (1950) and F_3 (1951) Data

In 1950 the F_2 generations of each cross were grown in three parts, sown at 15-day intervals, which for want of a better term were called "replicates". Seeds from F_2 plants in each replicate were used to produce the F_3 in 1951, maintaining the distinction of the replicates but sowing all on the same day. The date of sowing of the 1950 F_2 "B replicates" and of the 1951 F_3 lines was very nearly the same. Consequently, data from these materials have been used to compute correlation coefficients (r) measuring the correlation of days to heading of individual F_2 plants and mean days to heading of related F_3 lines, as follows:

Atsel × Tulare: r =0.725, P=0.001
Atsel × Bonneville: r 0.74=7, P=0.001
Atsel × Frontier: r = 0.665, P=0.002
Montcalm × Atsel: r = 0.835, P=0.001
Montcalm × Beecher: r = 0.897, P=0.001
Montcalm × Tulare: r = 0.956, P=0.001
Frontier × Bonneville: r = 0.856, P=0.001.

These results indicate that the F_2 genotype and the F_3 "average genotype" (a statistical equivalent of the F_2 genotype) consistently expressed the same phenotype with relatively little over-all disturbance by the environment.

Genetic Analysis in the F_3

(a) *Analysis on the Basis of the Ratio of Segregating to Non-segregating Lines*

This analysis is simple and has the advantage of having a non-arbitrary basis. (See section on "Materials and Methods"). Variances were calculated for each F_3 line in each "replicate" and for the two rows of each parent present in the replicate. (Replicate A consisted of 15 and replicates B and C each of 20 F_3 lines). Those F_3 lines that gave variances greater than those of either parent were classified as segregating, those with variances equal to or less than parental variances were classified as non-segregating.

The results are given in Table 2, together with "goodness-of-fit" tests of observed frequencies to frequencies expected on the basis of hypothesized ratios.

An excellent fit to the 1:1 ratio, which denotes a monohybrid, is obtained in the case of Atsel × Tulare. In all other crosses, high probability values are obtained for correspondence to the 3:1, the dihybrid ratio of segregating to non-segregating lines.

TABLE 2.—TEST OF GOODNESS-OF-FIT OF OBSERVED FREQUENCIES OF SEGREGATING AND NON-SEGREGATING F_3 LINES TO EXPECTED FREQUENCIES IN HYPOTHESIZED 1:1 (ATSEL × TULARE ONLY) AND 3:1 RATIOS

Cross	Segregating no.	Non-segr. no.	χ^2	P
Atsel × Tulare	25	30	0.455	0.50
× Bonneville	40	15	0.152	0.70
× Frontier	43	12	0.297	0.60
Montcalm × Atsel	45	10	1.364	0.25
× Beecher	37	18	1.752	0.15
× Tulare	43	12	0.297	0.60
Frontier × Bonneville	43	12	0.297	0.60

There are difficulties, however, in drawing definite conclusions. Take, for example, the cross Atsel × Tulare. Data in Table 1 suggest transgressive segregation, and data in Table 2 suggest a monohybrid condition; but it is theoretically impossible to have transgressive segregation in a monohybrid. It also seemed unlikely that six out of seven crosses would turn out to be dihybrid, although it proved fairly easy to assign to the six parental varieties genotypes that were consistent with this result.

As a further step in the analysis, the data of the seven crosses were examined with the view of taking both $\bar{D}$ and V into account and of detecting a fuller range of phenotypes among the segregating distributions.

(b) *Analysis on the Basis of $\bar{D}$ and V Values in F_3 Lines (F_2 Progeny Distributions)*

The theoretical basis for this analysis has been outlined in the foregoing section on "Materials and Methods".

The Atsel × Bonneville data have been selected for outlining the analysis in some detail because it has been demonstrated, in the section on the F_1 and F_2 generations, that inheritance in this cross is determined by additive (increasing) alleles. The analytical outline is given in Table 3 together with relevant data for the parents and P value from the χ^2 test.

TABLE 3.—DETAILED OUTLINE OF THE ANALYSIS OF DATA FROM F_3 LINES ATSEL × BONNEVILLE, ASSUMED TO BE DIFFERENTIAL FOR ADDITIVE ALLELES AT TWO LOCI

F_2 individuals				F_3 lines (F_3 progeny distributions reflecting F_2 genotypes)					
Genotype	No. incr. alleles	Ratio	Expected frequency	Observed frequency	Breeding type	Av. no. incr. alleles	$\bar{D}$ (range)	Segregation	V (range)
1 $a_1a_1b_1b_1$	4	1	3.44	3	Hom. late	4	60.1–61.4	None	2.5–5.5
2 $a_1a_1b_1b$ 2 a_1a b_1b_1	3	4	13.76	14	Het. late	3	56.7–62.0	1:2:1	4.3–10.1
4 a_1a b_1b	2	4	13.76	13	Het. int.	2	54.4–57.8	1:4:6:4:1	5.6–12.1
1 a_1a_1b b 1 a a b_1b_1	2	2	6.88	8	Hom. int.	2	53.3–55.8	None	1.8–5.3
2 a_1a b b 2 a a b_1b	1	4	13.76	13	Het. early	1	49.6–54.7	1:2:1	4.1–13.1
1 a a b b	0	1	3.44	4	Hom. early	0	51.4–53.6	None	1.1–3.9
Parental data	Atsel Bonneville		— —	—	Hom. early Hom. late	0 4	49.0–51.4 60.5–64.1	None None	2.2–5.5 2.7–4.7

55.04 55 $\chi^2 = 0.377$, P =0.99

TABLE 4.—PARENTAL AND F_3 HYBRID DISTRIBUTIONS IN ATSEL × BONNEVILLE, WITH DATA ON DAYS TO HEADING (D.T.H.) OF INDIVIDUAL PROGENETORIAL F_2 PLANTS, AND $\bar{D}$, V AND N VALUES OF THE F_3, TOGETHER WITH ASSIGNED BREEDING TYPES

Material	Days to heading of F_3 plants and parental varieties																									1950 D.T.H.	1951 $\bar{D}$	V	N
	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71				
Atsel			1	16		4	3		1	1																		45	49.0	3.088	26
				4	4	8	10	2	2	1																		45	50.4	2.255	31
Bonneville												1	1	2	9	2	4	12	2	2								53	60.6	3.839	35
													1	6	9	2	4	10	2		1	1						53	60.5	4.49	36
F_3 (1)								4	11	19	9	3	1			1	(hom. int.)											48	54.1	1.97*	48
(2)						3	5	15	4	11	6	1	2		1		1	(het. early) **										48	53.3	4.88	49
(3)						1	8	6	1	5	7	5	4	2	1	1		1	(het. int.)									49	54.4	7.99	42
(4)											6	4	4	2	13	3	2	7	2	(het. late)**								49	58.8	6.04	43
(5)		1	7		6	6	5	17	9	7		1	(het. early)															49	51.2	5.28	59
(6)						4	6	2	3	10	15	8	2	(hom. int.)														50	53.9	3.95*	50
(7)						6	6	9	2	14	7	7			1	(hom. int)												50	53.3	4.37*	52
(8)			1		3	7	3	6	4	3	2	(hom. early)																50	51.5	3.90*	29
(9)						1	5	4	11	6	9	8	1	5	1	(het. early)												51	54.3	4.77	51
(10)									2	1	4	11	5	4	4			5	1	(het. late)								51	57.4	6.79	37
(11)						2	1	9	6	4	7	8	5	3	2	1		4	(het. int.)									51	55.2	9.43	52
(12)				2		4	1	6	11	10	8	6		1	(hom. int.)													52	53.4	4.37*	49
(13)										1	12	4	3	8	6	5		7	6	1	1	1	(het. late)					53	58.8	10.09	55
(14)							1	1	4	4	10	16	7	2	2	6		2	(het. int.)									53	56.3	5.56	55
(15)												4	4	9	3		2	14	8	(hom. late)								54	60.1	5.48	50
(16)											7	5	6	2	7	6	2	7	3	4		1	(het. late)					54	59.3	9.26	50
(17)										4	6	6	2	7	6	3	1	1	2	(het. late)								54	57.5	6.15	38
(18)											2	2	3	4	3	5		7	6	1		3	(het. late)					55	60.4	9.34	36
(19)									1		8	9		7	2	1	1	4	2	(het. late)								55	56.9	7.43	48
(20)												1	1	2	4	7	3	11	8	1	3		1	(hom. late)				55	61.4	5.13	42

* Hybrid V values less than those of the parents

** The het. early type is only moderately early, and the het. late type is only moderately late

The nature of the analysis should be clear from the outline. Special points to be noted are: *first*, the relation between breeding type, average number of increaser alleles and the values of $\bar{D}$; and *second*, the relation between degree of segregation and the values of V. These were the main points scrutinized in deciding on which F_2 genotype to assign as being progenetorial to a given F_3 line; they are, therefore, main points in the reliability of the analysis.

The correspondence between observed and expected frequencies in Table 3 is very close. How reliable was the basis for determining the observed frequencies? This question is one of assessing each individual distribution in terms of its best fit to the various breeding types expected on the basis of a number of possible hypotheses. It is, therefore, important to demonstrate the nature of the distributions involved and the manner in which breeding types were assigned to them.

Table 4 gives the complete data compiled for the B "replicate" of Atsel × Bonneville. To show the practical thinking involved in assigning breeding types to distributions, let us consider distribution Number 1. It has a very low V and must be considered homozygous; $\bar{D}$ approximates that of the F_1 (which averages two increaser alleles) and we must, therefore, assume two increaser alleles; these conditions satisfy only the homozygous-intermediate breeding type. Considering distribution 2, with its moderately low V and $\bar{D}$, we find that it most closely satisfies the requirements of the heterozygous-early type. Distribution 3, with a rather high V and intermediate $\bar{D}$, is classified as heterozygous intermediate. Note that distribution 13 was classified as heterozygous late in spite of a large V; this was done because of the value of $\bar{D}$ which was too large for the heterozygous-intermediate type. In a few cases such compromises, though hardly satisfactory, were necessary. Another example is provided by distribution 20, where a rather large V was accepted for the homozygous-late type because the very large $\bar{D}$ suggested the presence of four increaser alleles.

Considering all seven crosses studied, this replicate is fairly representative of the difficulties in assigning breeding types to distributions. Do the compromises invalidate the analysis? There is room for argument. Let us take into account two facts: *first*, that additive alleles were strongly indicated for the present cross (Table 1); *second*, that a dihybrid was strongly indicated by the ratio of segregating to non-segregating lines (Table 2). Unless rather unusual assumptions are made, the breeding types and ratios outlined in Table 3 must occur in a cross differential for additive alleles at two loci. Actually, such difficulties as occurred in assigning breeding types were no greater nor more frequent than were to be expected. And there were many reassuring aspects to the analysis as, for example, the consistent occurrence of the homozygous-intermediate type, with low V and intermediate $\bar{D}$, throughout the data of all crosses. Incidentally, the ratio of segregating to non-segregating lines among the F_3 lines in Table 4, by the method shown in Table 2, is exactly 3:1.

It will be worthwhile to give a further example of the practical situations met with in assigning breeding types, especially in relation to tendencies toward high variability within distributions which suggest transgressive

* Hybrid V values less than those of the parents
** The het. early type is only moderately early, and the het. late type is only moderately late

TABLE 5.—PARENTAL AND F_3 HYBRID DISTRIBUTIONS IN MONTCALM × ATSEL, WITH DATA ON DAYS TO HEADING (D.T.H.) OF INDIVIDUAL PROGENITORIAL F_2 PLANTS AND $\overline{D}$, V AND N VALUES OF THE F_3, TOGETHER WITH ASSIGNED BREEDING TYPES

Material		Days to heading of F_3 plants and parental varieties																									1950 D.T.H.	1951 $\overline{D}$	V	N
		45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70				
Montcalm															4	3	16	9	13	4	2							63	60.8	2.45	5
																	10	4	17	16		1		2				64	62.1	2.42	50
Atsel					4	7	24	6	8																			44	50.1	1.25	9
					1	3	13	5	1		1																	45	50.3	1.33	24
F_2	(1)						1				6	12	24	4	2	2	(ho	m.	int.)									45	55.7	1.94*	51
	(2)			3	6		4				2	2	8	6	19	12	4	1	(het	. int.	)							45	56.0	16.23	67
	(3)	1	3	4	4	4	16	11	4			(ho	m.	early	)													46	49.7	3.83	49
	(4)	3	2		7	2	10	7	2			2	3		4	1	(het	.	early)									46	51.0	14.47	43
	(5)			2	4	3	9	9	6		3	2	5		2	1	(het	.	early)									47	51.8	9.21	46
	(6)			1	2	2	5	7	8	4	6	3	3		6	1	2	1	(het	.	early)							47	53.4	11.71	51
	(7)	1	1		5		5	7		1	1	2		1		3	1	1	(het	.	early)							48	52.1	18.99	29
	(8)						7	9	8	3	5	2	(ho	m.	early)													48	51.9	2.35*	34
	(9)				3		1	6	3	2	1		1	2	2	2	2	1	(het	.	int.	)						49	53.9	16.67	26
	(10)					2	3	3	7	8	10	3	4		2	(het	.	early)										50	53.2	4.50	42
	(11)										4	6	9	6	14	2	7		3	(het	.	late)						54	57.4	4.41	51
	(12)							1	2	2	8	7	2		4	3	1	1		1	(het	.	int.	)					54	55.7	7.89	32
	(13)										6	9	18	3	5		2	(ho | m. | int.)									54	56.0	2.10*	43
	(14)						2	1	3	4	8	2	1	1	3	2			1	1	(het	.	int.	)					55	54.9	10.48	29
	(15)				1		3	1	3	2	5	1	2		3	2	1	(he	.	int.	)								55	54.2	10.84	24
	(16)															3	3	1	1	3	(ho | m. | late)						56	60.8	2.26*	11
	(17)									3	4	7	11	2	9	2	3			4	(het	.	int.	)					56	56.9	7.06	45
	(18)											1	3	2	12	8	3	3	4	5	1	(het | . | late)					58	59.4	5.17	42
	(19)												5	3	9	7	10	4	7	3	(het | . | late)						58	59.4	4.12	48
	(20)											2	1		5	4	3	4	12	7	2	1		2	1	(het | . | late)	59	61.3	8.20	44

* Hybrid V values less than those of the parents

segregation in extreme breeding types. The B "replicate" of Montcalm × Atsel has been selected for this example because it includes all breeding types in about the expected proportions. The full data are presented as Table 5.

In this cross there are several instances of extensive variability which made it difficult to distinguish between heterozygous-early and heterozygous-intermediate and between heterozygous-intermediate and heterozygous-late types of distributions. In this replicate the difficulties involved heterozygous-early and heterozygous-intermediate. Distributions 4, 5, 6, and 7 are nearly as early as the early parent, but have V values appropriate to the heterozygous-intermediate type. All were designated heterozygous-early on the basis of $\bar{D}$ values. Questions arise. Should not distribution 6 be designed as of the heterozygous-intermediate type? Indeed, the over-all picture, which will be discussed in relation to Table 7, indicates that the distributions tends to be skewed toward the left (earliness) and that observed heterozygous-early frequencies are too large at the expense of heterozygous-intermediate frequencies which in turn are too large at expense of heterozygeous-late frequencies. That this skewing to the left may be environmental is suggested by shifts in the parental distributions in Table 5.

Apart from these difficulties in distinguishing between heterozygous-early and heterozygous-intermediate breeding types, the analysis of Table 5 is quite straightforward and satisfactory.

Before leaving Tables 4 and 5 it should be noted that the successive lines were grown from successively later F_2 parents. This produces successively larger $\bar{D}$ values with good consistency, most notable exceptions being distribution 4 in Table 4 and distribution 2 in Table 5. Data on days to heading of individual F_2 plants were not considered to be sufficiently reliable to be useful in assigning breeding types.

In Table 3 a detailed outline was given of analysis of data from F_3 lines of the Atsel × Bonneville cross. The analysis was based on the assumption of additive alleles at two loci, postulating six breeding types with 4, 3, 2, 2, 1 and 0 increaser alleles and in the ratio of 1:4:4:2:4:1. (The first of the types with two increaser alleles is heterozygous at both loci, the second is homozygous).

This form of analysis is extended in Table 6 to data from Atsel × Tulare, Atsel × Frontier and Frontier × Bonneville, and in Table 7 to data from crosses of Montcalm with Atsel, Beecher and Tulare.

Considering the Atsel × Tulare cross first, we find that there is no evidence of partial dominance of lateness nor of transgressive segregation for lateness, both of which had been suggested by the F_1 and F_2 data of Table 1. Indeed, such transgressive segregation as might be indicated would be for early types.

Other points to be clarified respecting the Atsel × Tulare cross are the contrary suggestions that it is a monohybrid in Table 2 and a dihybrid in Table 6. The former case arose from the rigid application of the condition that only a distribution with higher V than the parent may be considered as segregating. Since this is an early × early cross, the degree of segregation is relatively small and, with relatively large parental V values,

TABLE 6.—ANALYSIS DATA FROM F_2 LINES OF ATSEL × TULARE, ATSEL × FRONTIER AND FRONTIER × BONNEVILLE, EACH CROSS ASSUMED TO BE DIFFERENTIAL FOR ADDITIVE ALLELES AT TWO LOCI

Expected frequencies	Atsel × Tulare			Atsel × Frontier			Frontier × Bonneville		
	Obs. freq.	$\bar{D}$ (range)	V (range)	Obs. freq.	$\bar{D}$ (range)	V (range)	Obs. freq.	$\bar{D}$ (range)	V (range)
3.44	4	52.8–53.8	1.3– 2.5	2	61.2–65.4	4.8– 8.9	2	66.0–66.3	3.0– 3.6
13.76	11	51.1–53.8	2.6 –6.9	13	59.0–61.0	5.6– 9.7	10	65.8–69.8	5.2 –40.0
13.76	19	50.1–51.4	3.1 –9.6	14	56.4–60.0	7.2–24.4	14	61.5–67.5	16.8 –58.9
6.88	7	50.6–52.4	0.66–2.5	10	54.1–58.5	2.0– 4.1	10	64.1–64.9	1.7 – 7.0
13.76	13	47.3–49.9	2.2 –5.7	12	54.1–56.7	5.0– 9.4	15	58.3–62.8	7.5 –17.4
3.45	1	46.2	1.3	4	52.4–53.9	3.2– 5.0	4	55.1–58.3	0.84– 5.3
——	—	(A)48.5–50.9	(A) 1.8 –4.8	—	(A)48.5–51.4	(A) 1.7– 5.5	—	(F)63.5–67.7	(F)1.8 – 2.9
Parental data	—	(T)49.3–53.0	(T) 0.87–4.6	—	(F)64.1–67.4	(F) 1.0– 3.1	—	(B)61.6–64.4	(B)1.6 – 4.3
55.04	55	$\chi^2 = 4.375$, P = 0.50		55	$\chi^2 = 2.340$, P = 0.80		55	$\chi^2 = 3.212$, P = 0.65	

the estimated number of homozygous types was too high. When subjected to the analysis of Table 6, all six of the postulated breeding types were distinguished, and it is believed highly probable that they occurred approximately in the frequencies observed. On the basis of these frequencies, there are 43 segregating lines and 12 non-segregating lines. The test for goodness-of-fit to the 3:1 ratio gave $\chi^2 = 0.297$, $P = 0.60$. The conclusion is that, although the range of segregation is relatively small, inheritance of earliness in Atsel × Tulare is determined by additive alleles at two loci.

Atsel × Frontier results are quite consistent with expectations based on the hypothesis, as were the F_2 results in Table 1.

Frontier × Bonneville, a late × late cross, gave F_1 results (Table 1) which suggested partial dominance of earliness. There is little suggestion of this in the data of Table 6, which were quite consistent with expectations.

Turning now to consideration of the Montcalm crosses of Table 7, we must take into account the rather strong indications of transgressive segregation for, and partial dominance of, earliness in F_2 data of Montcalm × Beecher in Table 1. The averaged data from F_3 lines in Table 7 bear out these observations, although to a somewhat moderated degree. Four of the hom. early types observed had $\bar{D}$ values lower than those of Beecher (46.1, 47.1, 48.0 and 48.1 compared to 48.5 and 51.7). There is also a noticeable shift of frequencies toward the earlier categories, which produces a barely satisfactory fit to the hypothesized ratio ($P=0.15$). However, these disparities are considered to be somewhat minor variations in a mode of inheritance otherwise consistent with expectations.

In the case of Montcalm × Atsel, the suggestion of transgressive segregation for lateness shown by F_2 data in Table 1 is not borne out in Table 7. Indeed any significant shift of frequencies that might be considered would be, as in Montcalm × Beecher, in the direction of the earlier categories. It will also be useful to consider the difficulties of distinguishing between het. early and het. int. types, which was met with in the analysis of Table 5. In that connection it was suggested that, because of a skewing of all distributions toward earliness, het. early frequencies were too large at the expense of the het. int. frequencies which in turn were too large at the expense of the het. late frequencies. The observed frequencies are 17, 17 and 8, respectively, for these categories. Note that a shift of 3 frequencies from each of the earlier to the later categories would give 14, 14 and 14 respectively. However this may be, the data for Montcalm × Atsel are, as given, reliably consistent ($P = 0.40$) with expectations based on the hypothesis.

Little need be said about Montcalm × Tulare (for which the F_2 population, given in Table 1, was very small) other than to note that there were no particular difficulties in the analysis, and that the P value of 0.70 indicates a very good fit between observed and expected frequencies in the analysis.

GENERAL DISCUSSION

All discussion pertinent to specific crosses has been given in the previous section. There remains to give a brief, evaluatory review of the method of analysis. It is realized that this evaluation might be, at least in part,

TABLE 7.—ANALYSIS OF DATA FROM F_3 LINES OF MONTCALM CROSSES WITH ATSEL, BEECHER AND TULARE, EACH CROSS ASSUMED TO BE DIFFERENTIAL FOR ADDITIVE ALLELES AT TWO LOCI

Expected frequencies	Montcalm × Atsel			Montcalm × Beecher			Montcalm × Tulare		
	Obs. freq.	$\bar{D}$ (range)	V (range)	Obs. freq.	$\bar{D}$ (range)	V (range)	Obs. freq.	$\bar{D}$ (range)	V (range)
3.44	2	60.8–70.4	2.8– 5.7	3	57.4–59.6	1.6– 5.0	3	56.3–59.8	1.2– 3.8
13.76	8	57.4–68.1	4.4–38.3	9	54.3–58.1	3.2–13.4	10	55.9–59.2	8.8–20.6
13.76	17	53.6–56.9	7.0–30.0	15	51.1–53.5	9.1–23.3	15	50.1–55.7	10.1–25.2
6.88	6	52.8–57.0	1.0– 4.8	6	52.8–55.6	1.0– 2.8	7	49.2–52.7	1.2– 6.4
13.76	17	50.2–54.6	4.2–18.9	14	49.2–51.8	7.5–16.7	15	47.1–51.8	5.3–27.0
3.44	5	48.9–52.4	2.3– 4.6	8	46.8–50.3	1.6– 3.6	5	43.7–49.9	1.2– 3.0
——	—	(M)59.4–62.1	(M) 2.1– 3.6	—	(M)59.0–60.9	(M) 1.6– 4.6	—	(M)59.0–60.6	(M) 1.3– 5.1
Parental data	—	(A)49.6–50.8	(A) 0.2– 3.7	—	(B)48.5–51.7	(B) 0.3– 2.5	—	(T)47.6–51.5	(T) 1.1– 3.6
55.04	55	χ^2 = 5.319, P = 0.40		55	χ^2 = 7.936, P = 0.15		55	χ^2 = 1.976, P = 0.70	

a defence of the analytical method used, for some disagreement might well be anticipated.

When genetic analyses of the present data were first approached, consideration was given to the use of the strictly biometrical method of Mather (6). This method properly applies to continuous variation of a nature such that distinct phenotypes cannot be visually distinguished nor directly counted in the Mendelian manner. It was applied to the F_1 and F_2 generations in the present study, and was useful in indicating the additive nature of the alleles in some crosses; but it was rejected upon finding, in the F_3, that distinct phenotypes could be distinguished and counted in the classical Mendelian manner. Under these conditions, it was felt that biometrical analysis had less to offer than the analytical method finally adopted.

The genetic analyses of the present study are largely based on, and indeed had their origin in, the fact that six distinct breeding types were distinguishable among frequency distributions of F_3 hybrids arranged in days-to-heading categories. Each breeding type could be determined by characteristic variance (V) and mean days-to-heading ($\bar{D}$) values. On the basis of breeding-type data, it was hypothesized that parents differed by additive, increaser alleles at two loci in each cross, for example, $a_1a_1b_1b_1$ (late) $\times$ aabb (early). Theoretically, such a cross produces six breeding types: late-, intermediate- and early-homozygous types; and late-, intermediate- and early-heterozygous types; the ratio being 1:2:1:4:4:4 (rearranged 1:4:4:2:4:1 in the text). Observed frequencies being in good agreement with these expectations, it was concluded that the hypothesis accounted satisfactorily for the main features of inheritance. More or less minor discrepancies, especially in some crosses, are considered to be due to modifying genes (possibly polygenes), the nature of which could not be determined by the method used.

Concerning the second objective, to provide genetic guidance in breeding for earliness in barley, it has been shown in this study that types as early or earlier than the early parent, and apparently homozygous for earliness, occurred regularly at a rate of about 1 in 16 segregates. This favourable discovery should be very encouraging to plant breeders, and should lead to increased interest in the breeding of superior, early barleys.

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THE EFFECTS OF 2,4-D SPRAY DRIFT ON SUNFLOWERS[1]

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ABSTRACT

At Saskatoon the effects of drift spray of the butyl ester, low volatile ester, and amine formulations of 2,4-D on growing sunflowers all resulted in distortion of the leaves and stems, and malformed heads. Effects decreased with distance but were clearly discernible at 120 rods. With a wind velocity of 9 miles per hour after 1 hour of spraying the total seed yield was reduced by 42, 38, and 37 per cent for ester, low volatile ester, and amine, respectively. Seed quality decreased with proximity to the sprayed area.

Similar damage occurred at Morden when low volatile ester of 2,4-D was applied adjacent to a breeding nursery.

INTRODUCTION

Damage to sunflowers from 2,4-D drift has been reported for several years in the Morden and Altona districts of Manitoba. It seemed desirable to assess the extent of this damage. It was decided to conduct the experiments at the Forage Crops Laboratory, Saskatoon, because sunflowers are not grown commercially in the Saskatoon area and thus it was possible to carry out tests without endangering farm crops. A similar problem in sweet clover has been reported by Greenshields and White (2). They found that drift of 2,4-D butyl ester reduced the seed yield of sweet clover by as much as 80 per cent at close range and from 25 to 30 per cent at 96 rods. Plant distortion was evident also at 96 rods. In a review by Friesen (1) some of the problems related to the use of weed control chemicals, particularly droplet size in relation to spray drift and volatilits of 2,4-D formulations, are clearly stated.

MATERIAL AND METHODS

In the spring of 1955, about 350 seeds of the Advance hybrid were planted in 8-inch pots outdoors, between two greenhouses. One-half were planted on May 1 and the remainder on May 10. Both plantings grew well and appeared to be uniform.

On June 21, 1955, all plants were taken to a field of summerfallow near Saskatoon and arranged in three replicates consisting of four plants from each of the two plantings. The distance between the replicates was 2 rods. There were seven treatments. The plants were placed at right-angles to the wind at the six distances of 5, 10, 20, 40, 80 and 120 rods from the area to be sprayed. The seventh treatment was the check, which was taken upwind about 80 rods before the spraying commenced. The source of 2,4-D drift was provided by spraying parallel to the plants, starting 5 rods from the nearest treatment and working away from them. A tractor-mounted boom-sprayer was used. The sprayer was equipped with T-73007 nozzles and operated at a pressure of 30 lb. per square inch with a tractor

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speed of 4 miles per hour to deliver approximately 5 gallons per acre. Two formulations were used: (a) an alkanolamine salt of 2,4-D; and (b) a mixed ester of 2,4-D. Spraying with the amine formulation commenced at 9.00 a.m., June 21, 1955, and continued for about 1 hour until 80 oz. of acid equivalent had been expended. Between 15 and 20 acres were covered during the operation. The plants were collected and taken upwind. Plants from a different vehicle were distributed in the same pattern and spraying with the ester formulation commenced at 11.00 a.m., continuing about 1 hour until 80 oz. of acid equivalent had been used. During spraying the wind was steady from the northwest at from 11 to 14 m.p.h.; average temperature was 72° F., and average humidity 40.6 per cent. All plants were taken back to the greenhouse site where they were observed for the remainder of the summer.

In 1956, approximately 275 sunflower plants of a single hybrid were started in 8-inch pots on May 12 on the same site. They grew well and were quite uniform. An experiment was carried out on June 26, similar to that of the previous year. The differences were as follows:—There was only one stage of growth. A third formulation of 2,4-D low volatile ester (propylene glycol butyl ether ester acid) was used as well as the two formulations of the previous year. All check plants were taken to the field with the others and brought back before spraying was commenced. The wind velocity varied from 9 to 12 m.p.h. during the time of spraying. The average temperature was 70.4° F., and average humidity 31 per cent. When spraying was completed all plants were transplanted to a field plot. They were placed in the same relative position that they had occupied at the time of treatment, except that a row of check plants was included as the seventh treatment. There were 2 feet between plants and 4 feet between treatments. Care was taken to avoid disturbing the roots and soil during transplanting. When all plants had been transferred they were watered once.

Six yield trials of 25 entries each in six replicates were damaged by 2,4-D drift at the Experimental Farm, Morden, in the summer of 1956. The damage is believed to have resulted from spot-spraying along a hedge, approximately 20 rods from the nearest plots and 50 rods from the most distant plots. A low volatile ester form of 2,4-D was used. Wind velocity was rated at 3 on the Beaufort scale, or about 10 miles per hour, blowing toward the sunflowers. Operating pressures were 100 to 120 lb. per square inch, which would promote small droplet size. Mean temperature on the day of spraying was 62.5° F., and maximum 68° F.

EXPERIMENTAL RESULTS

In the 1955 experiment, approximately 4 days after spraying, distinct effect became evident in the form of rugose, leathery leaves, and curved stiff stems. The distortion was scored for each plant. The average ratings are given in Table 1. Examination of this table shows the mixed ester was more severe in its effect than amine. The distortion rating for ester ranged from 2.27 to 9.75, and for amine from 1.33 to 7.39. Of the 12 values for distortion obtained for each formulation, 9 were below 3.00 for amine and only 1 for ester. For both formulations the distortion was more severe on the younger plants.

TABLE 1.—DISTORTION RATINGS OF SUNFLOWER PLANTS RESULTING FROM TREATMENT WITH AMINE AND ESTER FORMULATIONS OF 2,4-D AS DRIFT SPRAY, SASKATOON, SASK., 1955

Distance from spray area	Average distortion ratings*			
	Ester		Amine	
	May 1st planting	May 10th planting	May 1st planting	May 10th planting
Rods				
5	5.8	9.8	4.9	7.4
10	4.6	8.4	2.8	3.8
20	3.9	8.0	2.5	2.4
40	3.7	8.2	2.4	2.3
80	2.3	5.9	1.7	2.4
120	3.9	6.9	1.3	1.4
Check	1.0	1.2	1.0	1.2

Distortion—includes curling of stems, rugoseness of leaves, leatheriness, and stiffness of stems. Score of 1 indicates no damage and a score of 10 eventual killing.

The effects on the flowering date and height of plant did not show a clear pattern. On the whole, flowering date was delayed except for the amine formulation on the May 10th planting. Amine increased height of plant in both stages. The ester reduced height in the younger plants, and also at the 5-rod distance with the older plants. The reduction was striking for both stages at the 5-rod distance.

Photographs of the 1955 plantings at Saskatoon illustrate the type of damage that occurred. Examination of Figures 1 to 6 shows that the ester produced more severe effects than amine. Not clearly evident in the illustration is the thick, leathery nature of damaged leaves as contrasted with thin, pliable condition in check plants, although this condition existed on nearly all damaged plants.

Seed yields were not taken from the 1955 planting because the effects of the pots and of position and shading from the greenhouse caused extreme variability.

In 1956 at Saskatoon the plants were transplanted to the open field in order to obtain a more uniform environment. Distortion was similar to 1955. The most seriously affected plants were a few days later maturing. Particular attention was given to yield of the seed and its characters. A summary of the seed yields expressed as a percentage of the check is given in Table 2.

TABLE 2.—AVERAGE YIELD OF SUNFLOWER PLANTS EXPOSED TO GROUND SPRAY DRIFT OF THREE FORMULATIONS OF 2,4-D DRIFT AT SIX DISTANCES FROM POINT OF SPRAYING, SASKATOON, SASK., 1956. (EXPRESSED AS PERCENTAGE OF CHECK)

Distance (rods)	5	10	20	40	80	120
Mixed ester	28.3	37.0	62.1	63.6	77.7	79.2
Amine	43.2	54.1	61.2	60.7	75.5	80.9
Low volatile ester	25.0	57.2	80.6	72.1	76.1	62.2

FIGURE 1. July 4, 1955. Check plant. Note formation of bud and smooth, well expanded young leaves. Slight rugoseness is evident on older leaves, probably due to trace amounts of 2,4-D prevalent in the air at Saskatoon during the spraying season.

FIGURE 2. (*Upper right*) July 4, 1955. Amine at 5 rods, showing prominent rugoseness of older leaves, bow in the stem, and in the younger leaves distortion of petiole and lack of normal expansion.

FIGURE 3. (*Lower right*) July 4, 1955. Ester at 5 rods. Rugoseness is apparent on older leaves. Young leaves have ceased active growth; the one at the lower centre showing extreme distortion and longitudinal rupture of the petiole.

FIGURE 4. July 4, 1955. Amine at 120 rods, showing petiole distortion and lack of expansion in young leaves.

FIGURE 5. July 4, 1955. Ester at 120 rods. Note extreme rugoseness of older leaves and small size of younger leaves and bud as contrasted with the check in Figure 1.

FIGURE 6. July 26, 1955. Ester at 10 rods. Dwarfing occurred at the 5-rod distance, but at 10 rods and farther a long thick stem developed with small, extremely distorted leaves, giving the appearance of a large petiole associated with a distorted secondary leaf appearing from the original leaf axil.

The 1956 planting was placed in the field in a split plot design with three replicates. Analyses of the yield data showed that distances were significantly different with a P-value of less than .01. Formulations were significantly different with a P-value of less than .05. The amine and low volatile ester forms were slightly less damaging than common mixed ester of 2,4-D. There was no significant difference between low volatile ester and amine. The interaction between formulation and distance was not significant, indicating all formulations drifted with equal severity.

The reduction in seed yield was due to small head-size, rows of empty "seeds" in the heads, and lighter weight of the seeds per thousand. Heads of plants closer to the spray area were smaller in diameter and many of the disk flowers failed to open. The number of seeds per head was much less and the seeds were wedge-shaped, containing very little meat with a high percentage of hull. This would contribute to a low oil percentage. Seed weights averaged 46 to 53 grams per thousand in the 5-rod treatment, 61 to 63 in the 20-rod treatment, and 65 grams per thousand in the checks.

In the 1956 experimental plots at Morden, the damage from the spray drift of low volatile ester at 20 to 50 rods seemed to be insignificant when first observed. However, when the plants flowered it was noted that rings of disk flowers failed to open or opened abnormally in many heads, thus causing a portion of them to be sterile. This type of injury is illustrated in Figures 7, 8, and 9. At the bloom stage, damage was rated on a 0 to 5 scale, 0 indicating no positive damage as judged by leaf and flower appearance, and 5 the most severe damage. Ratings were given after a brief visual examination of the whole plot. Each plant was not examined separately.

Marked differences in rating occurred among replicates and among varieties. In the most severely damaged test mean ratings of the varieties varied from 1.00 to 5.00. In the least damaged test the variation was from 0.0 to 2.8.

In an effort to remove the effects of varieties on yield, and thus demonstrate an actual reduction in yield due to the 2,4-D injury, the individual plot yields were adjusted so that the replicate totals within each test were the same. Variation within a test after this adjustment could be attributed to environment, variety, or 2,4-D injury. The differences in yield of paired plots, differing by the same amount in 2,4-D injury rating, but chosen from within varieties, were then subjected to the "t" test. In choosing the pairs a given plot was not allowed to appear in more than a single pair.

TABLE 3.—PLOT YIELDS OF SUNFLOWERS IN RELATION TO INCREASED AMOUNTS OF 2,4-D INJURY, MORDEN, MAN., 1956

Injury rating	0	1	2	3	4	5
Number of plots	220	219	146	148	102	65
Mean yield (lb./ac.)	1926	1924	1896	1691	1473	1179
Yield in per cent	100	99.9	98.4	87.8	76.4	61.2

TABLE 4.—REDUCTION IN YIELD SHOWN BY PAIRS OF SUNFLOWER PLOTS DIFFERING IN 2,4-D INJURY, THE PAIRS FROM WITHIN VARIETIES AFTER ADJUSTMENT OF PLOT YIELDS TO GIVE IDENTICAL REPLICATE TOTALS, MORDEN, MAN., 1956

Difference in injury rating	1	2	3	4	5
Number of pairs	204	107	31	13	9
Reduction in yield (lb./ac.)	61	153	97	209	539
Per cent reduction	3.0	7.0	4.4	8.6	20.2
"t" value	1.57	2.88	0.84	1.43	2.70
"t" (P=.05)	1.97	1.98	2.04	2.16	2.31

Table 3 gives actual mean yields of all plots arranged by degree of injury. Those plots rated 0 on the injury scale yielded 1926 lb. per acre. The yield declined by 38.8 per cent down to 1179 lb. per acre as the injury rating increased to 5.

Table 4 gives yield data and values of "t" obtained from pairs of plots after adjusting the data. For all differences in the injury rating there was a reduction in yield. It ranged from 3 per cent for a difference of 1 on the injury scale up to 20.2 per cent for a difference of 5. The reduction was significant, P=.05, for the difference in injury rating of 2 and 5, demonstrating that 2,4-D caused a reduction in yield. From this information it may be concluded that the reduction in yield due to 2,4-D injury on these plots was between 20.2 and 38.8 per cent; the 20.2 per cent being the maximum value obtained independent of varietal and replicate effects, and 38.8 per cent the most severe reduction evident before removal of these sources of variation.

DISCUSSION

Some differences, though not striking, were apparent between the three formulations of 2,4-D used in these studies. The distortion effects as shown by data and photographs were more severe for the mixed ester than amine in 1955. The following year the yield data showed a significant difference between the mixed ester and each of the other formulations with the mixed ester having the more severe effect. The main differences between the formulations, based on yield data, occurred in the area closest to the source of spray. The mixed ester and low volatile ester forms reduced yield by 71.7 and 75.0 per cent, respectively, at the 5-rod distance, compared with 56.8 per cent for the amine form. At the 10-rod distance, the effect of the mixed ester was considerably more severe than that of the other two formulations, whereas at greater distances the differences were not as prominent.

Clear explanations for the differences in behaviour of the three formulations are not apparent. The effects were due to 2,4-D being conveyed to the plants in spray droplets or as gas following volatilization of the active constituent. Presumably the amount conveyed by the first means would

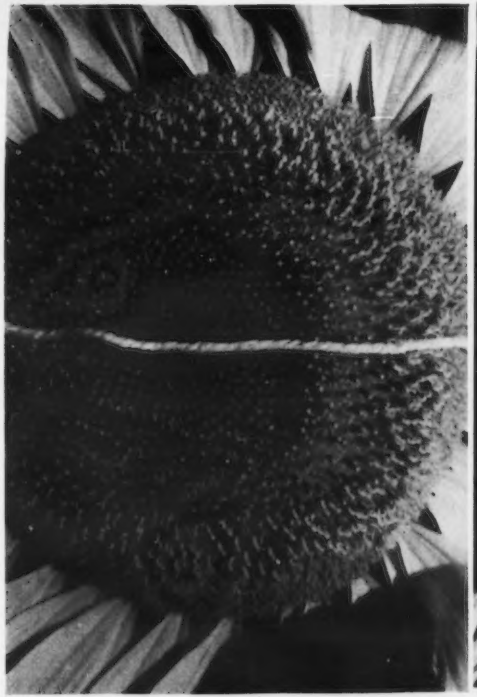

FIGURE 7. Morden, 1956. Normal head in bloom. Proceeding from outer edge note broad ray flowers followed by rings of disk flowers in which the anther tubes and stigmas have receded, a ring with bilobed stigmas exposed, a ring with anther tubes extended and unopened disk flowers in centre.

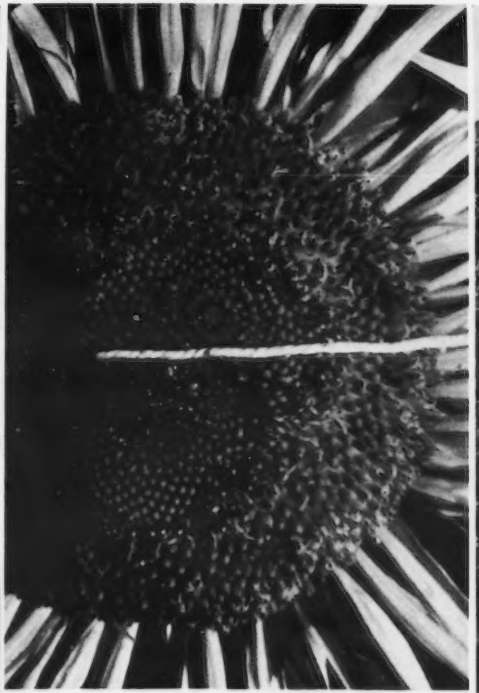

FIGURE 8. Modern, 1956. Head of plant which exhibited slight leaf injury from drift of low volatile ester. Contrast with Figure 7. Observe narrow ray flowers and irregularity of blooming pattern as shown by ring of disk flowers which has failed to open between two rings exhibiting irregular and partial opening only along with trilobed stigmas.

FIGURE 9. Morden, 1956. Mature head resulting from bloom such as illustrated in Figure 8. Note ring of small "seeds" corresponding to ring of unopened disk flowers in Figure 8. These "seeds" were empty hulls only.

have been uniform for all three formulations and it would have been deposited within a few minutes after the spray was applied, or as soon as the wind could have travelled the distance between the point of spraying and the plants. The amount deposited by the second means would vary with the volatility of the formulation. The mixed ester formulation is the most volatile, with the other two formulations being about equal. The time for the gases to reach maximum concentration or to remain at concentrations which are sufficient to injure sunflowers is unknown. Observations at Morden suggest that the gas persists in injurious amounts for several hours. In two occasions of injury to sunflower fields a common ester form of 2,4-D was applied to an adjoining grain-field while the wind was blowing away from the sunflower crop. The damage was attributed to a later change in wind direction toward the sunflowers which carried the fumes of 2,4-D over them. In one instance the interval between spraying and wind change was approximately 6 hours and in the other 24 hours.

The injury on the test plants also would be expected to bear some relation to the effectiveness of the three formulations as weed killers. In this regard they are usually rated in descending order as follows: low volatile ester, mixed ester, and amine.

With the foregoing thoughts in mind, it is believed that the injury incurred at Saskatoon in 1956 was largely due to 2,4-D deposited in spray droplets. At the close distance of 5 rods, where a relatively large amount of spray would have been deposited by droplet drift on the test plants, the damage was proportional to the effectiveness of the three formulations as weed killers. At greater distances, where differences were not so apparent, presumably the amount of 2,4-D deposited by drift was so small that differences in the formulations were not expressed. The exception of mixed ester at the 10-rod distance was an indication that some volatilization of this formulation was occurring but that it had not become serious enough to be an effective source of injury over the full width of the field. In retrospect it appears another treatment might well have been added to the experiment in a group of plants left in the field for several hours after spraying with the mixed ester, to determine if volatilization after spraying is an important feature in damage occurring from this form.

From the practical aspect the results of this study demonstrate conclusively that amine, mixed ester, and low volatile ester forms of 2,4-D cannot be used safely near a sunflower field when the wind is blowing toward it. The effects with all formulations were most severe at the close distances, but even at 120 rods from the point of application injury was clearly discernible and reduction in yield significant for all three formulations. Using the check yield as 100 per cent, the yield of a field of sunflowers 120 rods wide would have been reduced by 37 per cent by amine, 38 per cent by low volatile ester, and 42 per cent by mixed ester formulation under the conditions of the experiment at Saskatoon. The fact that these values could occur in a field 120 rods or approaching one-half mile wide stresses the serious effects that can occur from injudicious use of 2,4-D near commercial fields of sunflowers.

The results suggest that amine is the preferable formulation of 2,4-D when using this chemical near sunflowers, followed by low volatile ester. The mixed or more volatile forms of ester and use of high operating pressures when spraying with any form should be avoided.

ACKNOWLEDGEMENTS

All forms of 2,4-D used in the controlled experiments at Saskatoon were supplied by Chipman Chemicals Limited. The low volatile ester used at Morden was Dow Esteron 1010. The use of these chemicals does not constitute an endorsement of them.

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MAJOR AND MINOR ELEMENT STATUS OF HEALTHY AND UNHEALTHY ALFALFA

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ABSTRACT

A study was made of the major and minor element status of healthy and unhealthy alfalfa. Elements determined were calcium, magnesium, potassium, phosphorus, molybdenum, manganese, zinc and boron. Unhealthy alfalfa was found to be deficient in potassium and/or boron, the critical levels being 1 per cent and 20 parts per million respectively.

The levels of other major and minor elements were similar in both healthy and unhealthy plants. Available soil boron was apparently positively correlated with plant tissue boron.

INTRODUCTION

During the summer of 1955 a study was begun to determine, if possible, the cause or causes of poor alfalfa growth on a number of Nova Scotia farms. This work was initiated at the request of the Nova Scotia Soils and Fertilizers Committee, as a result of farmers asking the reason why their alfalfa crop was stunted, unhealthy and bore discoloured leaves.

A literature review indicated possibilities of boron and/or potassium deficiency as factors in producing unhealthy alfalfa tissue. Critical values reported for the boron content of alfalfa range from 6.9 to 23.0 parts per million (7). Beer *et al.* (1, 5) report that contents above 1 per cent potassium and below 2 per cent calcium produce maximum alfalfa yields. They also state that the total milliequivalents of calcium, magnesium and potassium are relatively constant. Therefore, a change in one would vary the other.

SAMPLE COLLECTION AND PREPARATION

Samples of alfalfa tissue and related soil samples were collected from 50 farms representing 8 counties. Healthy alfalfa stands were sampled to establish their nutrient level, thus enabling a comparison to be made between these and unhealthy samples. Samples were classified visually as healthy or unhealthy, green luxuriant growth being the criterion for healthy tissue, while the unhealthy samples showing subnormal growth were divided into three groups, depending upon the deficiency symptoms encountered.

Group A included samples showing white marginal spotting as well as yellowing of the leaves.

Group B included those samples showing particularly severe yellowing of the leaves.

Group C samples showed white marginal spotting of the leaves as the main deficiency symptom.

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Samples were collected in August at the time of a second cutting. Alfalfa plants were cut 3 inches above the ground to lessen the chances of contamination. Each field was sampled by walking into the area 50 paces and taking the first plants, then moving the same distance to the right or left and sampling again. This method was followed until the entire area was covered. Soil samples were taken in the same manner.

All tissue and soil analysis results are calculated on an air-dry basis.

METHODS

Boron in alfalfa tissue was determined by the Hatcher and Wilcox carmine method (3) with the following modifications:

(i) The use of $Ca(OH)_2$ in place of CaO

(ii) Centrifuging at 2000 r.p.m. for 10 minutes rather than filtering.

Available soil boron was determined by the quinalizarin method of Berger and Truog (2).

Manganese was determined by the potassium periodate method in combination with H_3PO_4 (8). The following modifications were used:

(i) Heat gently for 30 minutes or boil for 5 minutes.

(ii) 12 gm. sample dry ashed, digested with 8 ml. of concentrated H_2SO_4 and 30 per cent H_2O_2 until colour becomes white, then filtered, made to 100 ml. volume and an aliquot used for the determination.

Potassium and magnesium were determined by the flame photometer method, using a Beckman DU spectrophotometer with flame attachment.

Molybdenum and zinc were determined spectrographically, using the concentration procedure of Mitchell (6).

Calcium was determined volumetrically by the oxalate-permanganate method (5).

Phosphorus was determined by the official A.O.A.C. ammonium molybdate method (5).

RESULTS AND DISCUSSION

The boron and potassium values for healthy and unhealthy alfalfa are given in Table I. For healthy alfalfa, the boron and potassium figures range from 19.9 to 57.1 parts per million, and 1.12 to 2.30 per cent respectively. These values are, in general, above the critical levels mentioned previously for healthy alfalfa growth.

With respect to unhealthy alfalfa, the results for group A are self-explanatory and indicate that these samples are below the 1 per cent potassium level, as well as being low in boron. Alfalfa such as this would be unhealthy, due to these two elements being lower than required for healthy growth. Results for group B samples indicate that the potassium contents are above the minimum level and comparable with the values found for healthy tissue. The boron contents, however, ranging between 10.1 and 16.0 parts per million, fall within the range denoting boron-deficient alfalfa. The results of analysis for group C indicate the reverse situation, boron being present in sufficient amounts while the potassium content is below the minimum level.

TABLE 1.—BORON AND POTASSIUM VALUES FOR HEALTHY AND UNHEALTHY ALFALFA

Healthy		Unhealthy			
B (p.p.m.)	K (%)		B (p.p.m.)	K (%)	
19.9	1.90		12.4	0.44	
33.2	1.73		15.8	0.56	
28.8	1.96		15.2	0.89	
31.3	2.05	A	11.2	0.55	
32.0	1.74		10.1	0.92	
29.8	1.27		16.0	0.93	
30.5	1.55		15.4	0.73	
53.4	1.41		12.3	0.96	
55.8	1.55	Average:	13.6		0.75
57.1	2.04		16.5	1.45	
29.6	1.76		19.0	1.66	
31.3	1.26		16.1	1.47	
31.0	1.43	B	13.4	1.02	
36.1	1.78		10.8	2.08	
43.3	1.58		14.7	1.69	
35.7	1.57		15.5	1.17	
40.3	2.08		17.0	1.23	
23.8	1.89	Average:	15.4		1.47
35.4	1.15		46.2	0.69	
20.6	1.12		23.8	0.53	
24.5	1.70		46.3	1.00	
24.8	1.12		51.2	1.02	
35.8	2.30	C	43.7	0.72	
36.8	1.41		31.3	0.78	
52.3	1.66		27.0	0.94	
			19.9	0.85	
			30.0	1.00	
Average: 34.9	1.61	Average:	35.5		0.84

TABLE IA.—STATISTICAL ANALYSIS "t" VALUES

Samples	K	B	P.01
Healthy – Unhealthy	6.56	5.21	2.79
A – B	5.10	0.48	3.25
B – C	4.07	5.82	3.25
A – C	0.64	5.34	3.25

Table I in general shows that the average values for boron and potassium in healthy alfalfa are 34.9 parts per million and 1.61 per cent respectively. Unhealthy alfalfa, on the other hand, averages 14.5 parts per million boron and 0.79 per cent potassium when either one or both of these elements are deficient. The groups A, B and C point out clearly that deficiencies of either one or the other, or both, elements were the cause of the unhealthy alfalfa problem existing in this province.

Statistical analysis, Table IA, of potassium and boron values shows there is a highly significant difference between healthy and unhealthy alfalfa tissue. The calculated "t" value for boron and potassium is 5.21 and 6.56 respectively, while the estimated "t" value for P .01 is 2.79.

TABLE 2.—AVERAGE AVAILABLE BORON VALUES AND pH RANGES FOR SOILS

Plant condition	B (p.p.m.)	pH Range
Healthy	0.48 ± .18*	6.03 – 6.83
Unhealthy Group A	0.20 ± .11*	5.83 – 7.05
Group B	0.19 ± .07*	6.09 – 6.88
Group C	0.50 ± .12*	6.03 – 6.92

* Average deviation from the mean

TABLE 3.—AVERAGE AND RANGE VALUES FOR OTHER MAJOR AND MINOR ELEMENTS IN ALFALFA

Element	Healthy		Unhealthy	
	Av.	Range	Av.	Range
Mo (p.p.m.)	0.45	0.01 – 1.50	0.62	0.13 – 1.50
Zn (p.p.m.)	13.6	4.2 – 29.5	12.3	5.1 – 23.0
Mn (p.p.m.)	65.5	36.2 – 127.6	68.6	30.8 – 122.2
Ca (%)	1.54	1.03 – 2.16	1.86	0.71 – 3.03
Mg (%)	0.43	0.19 – 0.68	0.52	0.19 – 0.91
P (%)	0.29	0.21 – 0.40	0.29	0.20 – 0.40

For unhealthy alfalfa statistical analysis showed a highly significant difference regarding potassium between A and B, and B and C groups, while for boron highly significant values were obtained between A and C, and B and C groups. For potassium "t" values of 5.10 and 4.07 were calculated, while for boron "t" values of 5.82 and 5.34 were obtained. The "t" value given for P .01 was 3.25. No significant difference was found between groups A and B for boron, or A and C for potassium.

The average available boron contents, average deviation from the mean, and pH values for the soils corresponding to the tissue samples, are listed in Table 2. From these results it can be seen that healthy alfalfa tissue and unhealthy potassium-deficient alfalfa, group C, were grown on soils containing above minimum amounts of available soil boron. Unhealthy boron-deficient alfalfa, groups A and B, were grown on soils low in available boron as reported by Ouellette and Lachance (7). The pH values of the soils, within the range encountered, have no apparent effect upon the condition of the alfalfa.

Table 3 shows the average values and the range covered for other major and minor elements determined. There is very little difference between the healthy and unhealthy samples regarding the elements molybdenum, zinc and manganese. The same is true for calcium, magnesium and phosphorus,

but it should be noted that the calcium and magnesium vary inversely as potassium. Also of importance is the fact that calcium is below the 2 per cent level as suggested by Bear (1, 5) for good alfalfa growth. It would appear from Table 3 that these elements are eliminated as factors in producing unhealthy alfalfa.

Finally, from work carried out during this survey, it was established that there are a number of farms producing unhealthy alfalfa due to boron and/or potassium deficiencies. Perhaps more serious is the fact that there are a number of farms growing alfalfa which does not show deficiency symptoms but where growth and yield are restricted because of insufficient amounts of the above elements. In fact, these plants may be being maintained on a mere subsistence level. More work is needed on this point. It is important that alfalfa growers follow a soil and tissue testing program to ensure maximum production of healthy alfalfa.

SUMMARY

(1) Healthy alfalfa plants contained 30-50 p.p.m. boron and above 1 per cent potassium.

Unhealthy alfalfa plants occurred when boron and/or potassium fell below these values.

(2) The content of other major and minor elements studied was similar in healthy and unhealthy plants.

(3) This work indicates there is a direct correlation between available soil boron and plant tissue boron.

ACKNOWLEDGEMENTS

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EFFECTS OF ORCHARD FUNGICIDES ON STORED McINTOSH APPLES[1]

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ABSTRACT

Storage observations were made from 1954 to 1956 on McIntosh apples from trees subjected to five fungicidal treatments. The fungicides were: phenyl mercuric acetate, captan, glyodin, sulphur paste and ferbam. Each treatment was replicated four times. For the 3-year period phenyl mercuric acetate was significantly associated with the least amount of fungal rotting and the maximum acid content in the fruit after storage at 32° F. for 5 to 6 months.

INTRODUCTION

Among the complex factors which influence the storage quality of fruit, pesticidal sprays have received little attention. Early studies by Hockey and Ward (5) showed that bordeaux sprays tended to increase the sucrose and total sugar content of apples. More recently, Garman *et al.* (7) reported that sprays of captan and arsenicals, respectively, tended to raise and lower the acid content of apples, whereas phenyl mercuric acetate (PMA) apparently had no effect in this regard.

According to Palmiter (8), the substitution of ferbam for flotation sulphur in cover sprays improved fruit colour.

Fungal rots in stored apples is an economic problem in the Annapolis Valley of Nova Scotia. This is particularly true of the rot caused by *Gloeosporium album* Osterw., an organism which is active under cold storage conditions. The disease caused by *Gloeosporium perennans* Zeller & Childs on Cox Orange apples in storage is stated by Austin (1) to constitute a threat to the apple industry in England. Hunman *et al.* (6) have found that captan sprays applied up to 3 weeks before picking have given promising results in the control of this disease. In 1956, Edney (3) reported partial control of storage losses from *G. perennans* by using ziram and captan during the growing season.

The aim of this investigation was to study the effect of various fungicides applied as cover and pre-cover sprays on the keeping quality of McIntosh apples in storage. The criteria of quality which were used included acid content of fruit, firmness, appearance and susceptibility to fungal rot.

MATERIALS AND METHODS

The fruit used in this work was obtained from an orchard near Kentville, N.S., in which a comparison of the effects of fungicides on mature McIntosh apple trees is being conducted by the Laboratory of Plant

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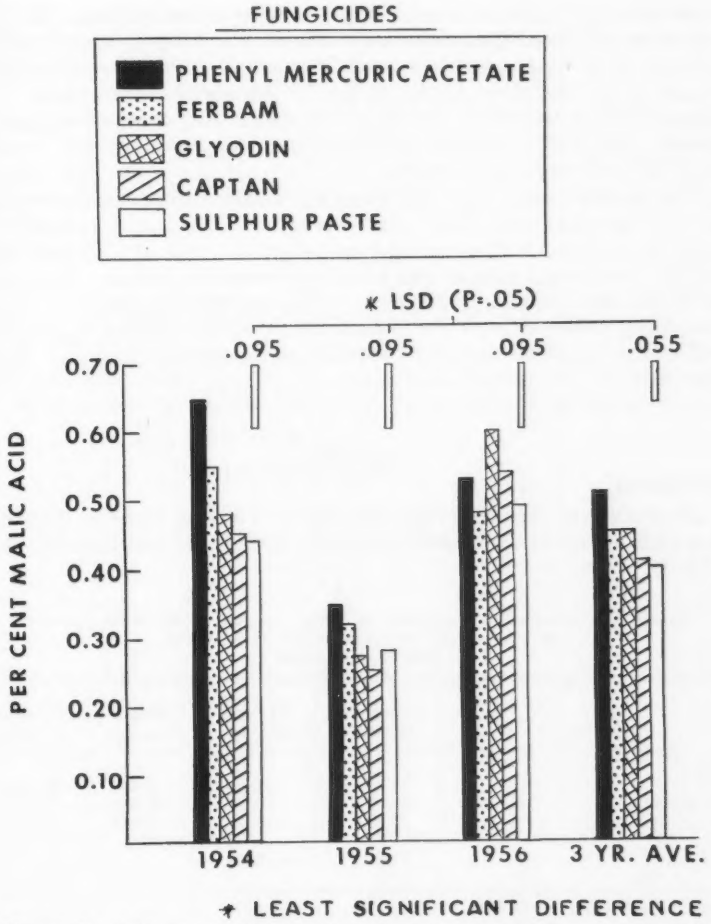

FIGURE 1. Acid content of McIntosh apples in storage as influenced by fungicidal sprays.

Pathology. The various fungicides used*, together with the concentration per 100 gallons of water for cover and pre-cover sprays, were respectively: captan, 2 lb., 1.5 lb.; ferbam, 2 lb., 1.5 lb.; sulphur paste (Magnetic 70), 9 lb., 6 lb.; glyodin, 1.5 qt., 1 qt.; PMA, 0.5 pt. in pre-cover and ferbam 1.5 lb. in cover applications. These treatments were applied to plots consisting of four trees in each of four randomized blocks. The quantity of dilute spray required to spray the trees to "run-off" averaged 6 and 9 gallons in the pre-cover and cover sprays respectively.

* P.M.A. (Erad), phenyl mercuric acetate, 10% (Green Cross Insecticides, Montreal, Que.) Captan (Captan 50-W), N-(trichloromethyl-thio)-4-cyclohexene-1,2-dicarboximide, 50% (Stauffer Chemical Co., New York, N.Y.) Glyodin (Crag Fruit Fungicide 341), 2-heptadecyl-2-imidozoline acetate, 34% (Green Cross Insecticides, Montreal, Que.) Sulphur paste, 69% (Magnetic 70) (Stauffer Chemical Co., New York, N.Y.) Ferbam (Fermate), ferric dimethyldithio-carbamate, 76% (DuPont Co. of Canada, Ltd., Montreal, Que.)

In 1954 four pre-cover and five cover sprays were applied. In 1955 all plots received an application of PMA at the delayed dormant stage following an 80-hour apple scab infection period. This was followed by four pre-cover and three cover sprays of the specified materials. The 1956 schedule consisted of two pre-cover sprays and three cover sprays. The entire orchard was harvested when the iodine test indicated that the core of the fruit was free of starch.

One bushel of fruit was then picked at random from one tree in each plot, the samples were stored immediately at 32° F. and removed for examination after 146 days in 1954 and after 170 days in 1955 and 1956. Estimation of fungal rotting was based on the entire sample. Ten fruits were tested for hardness by means of a Magness pressure tester, measurements being made on both the blushed and unblushed areas of the fruit. Total acidity was determined by a method previously described (2). Forty apples were cut for internal examination and this sample was also scored for appearance and quality by use of the ratings adopted by Hill *et al.* (4).

RESULTS

Acid Content

An analysis of variance of the data for total acidity revealed that there was a highly significant difference between treatments and between years (Table 1).

TABLE 1.—ANALYSIS OF VARIANCE OF ACID CONTENT OF McINTOSH APPLES AS INFLUENCED BY FUNGICIDAL TREATMENTS, 1954-56, INCLUSIVE

Source	Degrees of freedom	Sums of squares	Mean squares	Calculated F value
Blocks	3	.239		
Years	2	.7276	.3638	81.57**
Treatments	4	.0834	.02085	4.67**
Years × treatments	8	.0473	.00591	1.33
Error	42	.1872	.0046	
Total	59	1.0694		

** Significant at 1% level

TABLE 2.—ANALYSIS OF VARIANCE OF PERCENTAGE OF ROTS ON McINTOSH APPLES AS INFLUENCED BY FUNGICIDAL TREATMENTS, 1954-56, INCLUSIVE

Source	Degrees of freedom	Sums of squares	Mean squares	Calculated F value
Blocks	3	263.46		
Years	2	851.89	425.94	4.05*
Treatments	4	2917.36	729.34	6.94**
Years × treatment	8	1379.43	172.43	1.64
Error	42	4413.81	105.09	
Total	59	9825.95		

* Significant at 5% level
** Significant at 1% level

The spray treatment containing PMA was found to be associated with a significantly higher acid content than that found with the other treatments when all observations were averaged (Figure 1).

In 1954 the acid content of apples treated with PMA and ferbam was significantly greater than that of apples sprayed with captan and sulphur paste. The following year the apples treated with PMA contained significantly more acid than those sprayed with captan. In 1956 glyodin was associated with a higher acid content than either ferbam or sulphur paste.

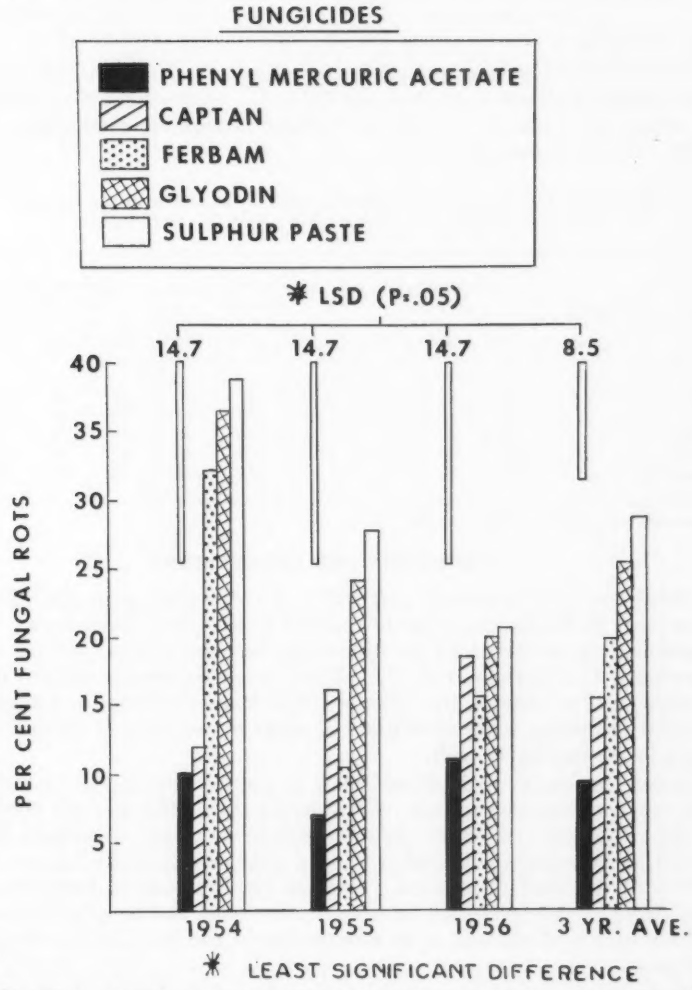

FIGURE 2. Fungal rot development of McIntosh apples in storage as influenced by fungicidal sprays.

Fungal Rots

The data obtained on the frequency of rotting were subjected to an analysis of variance (Table 2) and are shown graphically in Figure 2.

There was a highly significant difference between treatments over the 3-year period and a significant difference between years. The differences in response to treatment were particularly marked in 1954, but in 1955 only PMA differed significantly from glyodin and sulphur paste. In 1956 the differences were not significant. The dominant rot producing organisms in the samples were *Penicillium* sp., and *Gloeosporium album*.

Other Observations

The fungicidal treatments failed to produce any significant differences in the hardness of the fruit as shown in Table 3. Apples treated with PMA and captan were found to have the highest ratings for appearance and quality over the 3-year period.

TABLE 3.—INFLUENCE OF FUNGICIDAL SPRAYS ON THE STORAGE QUALITY OF MCINTOSH APPLES, KENTVILLE, N.S., 1954-56, INCLUSIVE

Spray treatment	Hardness, lb. pressure			Appearance, max. score 30*			Quality, max. score 100*		
	1954	1955	1956	1954	1955	1956	1954	1955	1956
PMA	10.8	11.8	12.6	30	25	28	86	61	80
Captan	10.3	11.8	13.2	29	24	28	85	69	79
Ferbam	10.5	10.8	12.8	25	24	25	80	56	74
Glyodin	10.4	11.0	12.9	26	20	25	82	60	76
Sulphur paste	10.4	11.5	13.2	25	22	27	80	60	76

* Scored according to methods of Hill *et al.* (4)

DISCUSSION AND CONCLUSIONS

This investigation showed that PMA was associated with the highest acid content in McIntosh apples in relation to the other treatments. No explanation can be offered for the differences between results obtained and those reported by Garman *et al.* (7). These investigators also showed that arsenicals tend to depress the mineral levels in apple leaves and suggest that foliar feeding or foliar absorption of spray materials may bring about changes in the quality of fruit.

Spray treatments with either PMA or captan gave good control of fungal rots on McIntosh apples. Considering that PMA was not applied after the calyx spray at which time the fruit had scarcely developed, it is doubtful if the control achieved was by a direct fungicidal effect on the organism at the time of infection. Control with PMA may, therefore, be due to either the physiological changes in the tissues as exemplified by the relatively high acid content or to a reduction in the amount of inoculum in the plots.

Observations on the appearance and quality of the fruit indicated that apples with the best ratings were from trees sprayed with PMA and captan.

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THE PERFORMANCE OF THREE GRASSES WHEN GROWN ALONE, IN MIXTURE WITH ALFALFA, AND IN ALTERNATE ROWS WITH ALFALFA[1]

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ABSTRACT

Crested wheatgrass, intermediate wheatgrass, and streambank wheatgrass, chosen for diversity of root type, were compared for yield and competitive ability when growing alone, in mixture with alfalfa, and in alternate rows with alfalfa. For each seeding method the order of the grass species yield performance was the same, but the magnitude of the yield difference varied by seeding methods. In pure stands the yield difference between the low and high producing grass was 50 per cent, in mixture with alfalfa 170 per cent, and in alternate rows with alfalfa 220 per cent. The total yield was greatest in alternate rows and smallest in pure grass stands (fertilized). In 1954 grass and alfalfa growing in alternate rows outyielded grass and alfalfa in mixed rows by 4 per cent; in 1955, by 10 per cent; in 1956, a dry year, by 33 per cent; and in 1957, an extremely dry year, by 137 per cent. The relative stand of alfalfa to grass was greater when growing in alternate rows as compared to mixed rows. This relationship held for all grass species but was less pronounced for streambank wheatgrass, the least competitive species of the three grasses.

INTRODUCTION

In the Prairie Provinces of Canada it is recommended that grasses be grown in mixture with alfalfa because hay yields are much greater than those obtained from grasses grown alone (2, 11, 16). Reports from other regions by other workers indicate similar results (9, 14, 18).

The usual way of establishing and growing mixtures is to drill the mixed seed into all rows. However, as early as 1942, Clarke and Heinrichs (3) reported that in trials where crested wheatgrass and alfalfa were seeded in alternate rows, the alfalfa performed better than where the grass and legume were seeded in the same drill rows. Lemmon and Hafenrichter (13) describe a method of seeding crops, including legumes, through alternate drill runs, but not for the purpose of studying its effect on yield and competition. Hughes (7, 8) reported that in England grasses and alfalfa in alternate rows for winter pasture provided intensive grazing, good animal gains, and that the botanical composition of the sward did not deteriorate as quickly as it did in mixtures. Chamblee and Lovvorn (1) in North Carolina found that grasses and alfalfa in alternate rows yielded less than when grown in mixture. Patterson and Law (15) in Washington reported that forage yields from seedings of grass and alfalfa in alternate rows, in most cases, were greater than yields of grasses and alfalfa seeded in mixture. Between the years 1950 and 1953, a few other workers (5, 15, 17, 18) employed alternate-row seeding of grasses and legumes for the purpose of studying grass strain evaluation techniques, erosion control, or effect on soil fertility.

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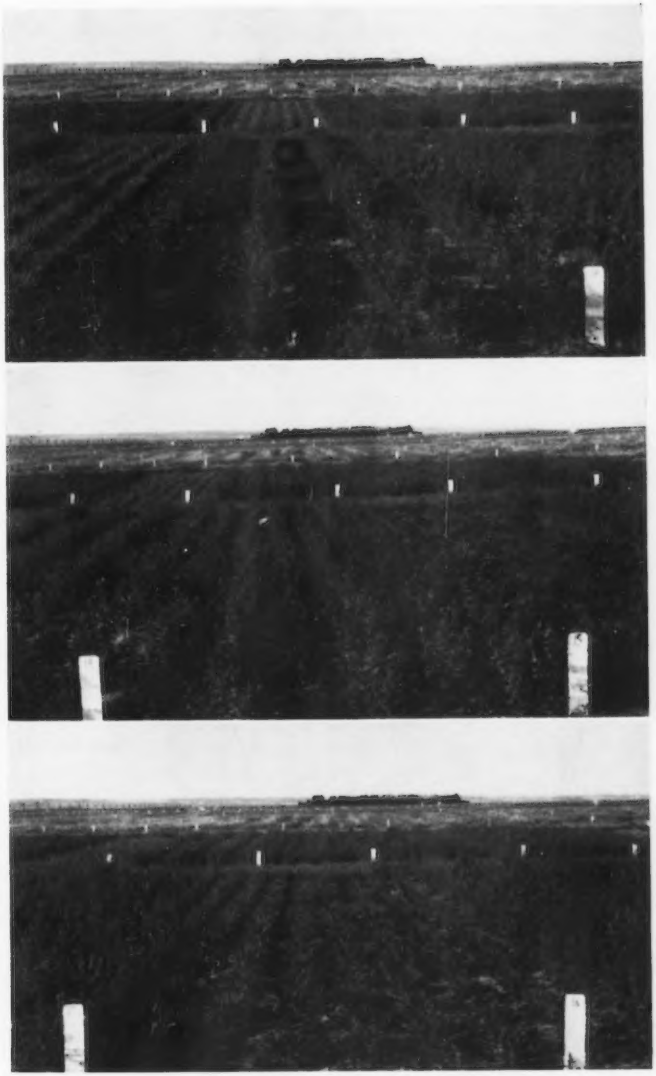

FIGURE 1. Excellent stands obtained in the establishment year. *From top to bottom:* intermediate wheatgrass in alternate rows with alfalfa; intermediate wheatgrass in mixture with alfalfa; and intermediate wheatgrass by itself.

The investigation reported in this paper was undertaken with a twofold objective: (a) to study the relative behaviour of three Agropyron species, crested wheatgrass *Agropyron cristatum* L. Gaertn., intermediate wheatgrass *Agropyron intermedium* (Host.) Beauv., and streambank wheatgrass *Agropyron riparium* Scribn. and Smith, when seeded in alternate rows, and in mixture, with Ladak alfalfa *Medicago media* Pers. and when seeded alone and fertilized; and (b) to determine the effect of methods of seeding grass and alfalfa on total yield and composition of the forage.

The three grasses were chosen because of their good adaptation to the climate of the area and for their diversity in type of root system. Crested wheatgrass is bunch rooted, intermediate wheatgrass is moderately rhizomatous, while streambank wheatgrass is strongly rhizomatous (2, 4, 6, 11, 12).

The climatic conditions at Swift Current, Saskatchewan, where the experiment was conducted, are semi-arid with a 35-year (1922-1956) average annual precipitation of 13.97 inches. The soil is medium textured and developed upon undifferentiated glacial till in the brown soil zone.

METHODS

The experimental design was a split plot with four replications. The grasses constituted the main plots and the seeding methods the sub-plots (Table 1). All sub-plots were 35 feet long, but widths varied from 6 to 8 feet. Where grass was seeded in pure stands or in mixture with alfalfa, the sub-plots consisted of six rows, spaced 12 inches apart, while in those sub-plots where grass and alfalfa were seeded in alternate 12-inch spaced rows, four rows of each were seeded to make sub-plots 8 feet wide.

The rates of seeding were such that approximately the same number of seeds were planted per row for each method of seeding (Table 1).

The test was planted with a hand V-belt seeder in May 1953. Moisture conditions were very good during the establishment year (Table 2) and excellent stands resulted (Figure 1).

Each fall the pure grass plots were fertilized with ammonium nitrate (33.5-0-0) at the rate of 100 lb. per acre.

TABLE 1.—METHOD AND RATES OF SEEDING

Main plots (grass species)	Sub-plots (seeding methods)	Pounds seed per acre		
		Grass	Alfalfa	Total
Streambank wheatgrass	Alternate rows: grass−alfalfa	3	2	5
	Mixture: grass+alfalfa	3	2	5
	Grass alone	6		6
Crested wheatgrass (var. Fairway)	Alternate rows: grass−alfalfa	3	2	5
	Mixture: grass+alfalfa	3	2	5
	Grass alone	6		6
Intermediate wheatgrass	Alternate rows: grass−alfalfa	6	2	8
	Mixture: grass+alfalfa	6	2	8
	Grass alone	12		12

TABLE 2.—ANNUAL PRECIPITATION DURING ESTABLISHMENT YEAR AND 4 HARVEST YEARS OF THE TEST

Month	1953	1954	1955	1956	1957
January	.93	.79	.82	1.23	.56
February	.95	.27	.41	.67	.25
March	1.50	.83	1.29	.42	.76
April	1.96	.93	2.76	.41	1.44
May	2.35	3.37	2.58	1.24	.13
June	3.77	3.51	1.67	3.65	1.84
July	.52	3.55	4.06	1.45	2.72
August	.51	2.77	.20	.33	1.30
September	2.06	2.74	.76	.78	.35
October	.28	.41	.49	1.60	1.16
November	.15	.43	1.23	.23	.83
December	.65	.11	1.04	1.14	.46
Total	15.63	19.71	17.31	13.15	11.80

TABLE 3.—4-YEAR FORAGE YIELD DATA AND VARIANCE ANALYSIS SUMMARY

Grass species	Seeding method	Dry matter yield—Tons/acre				
		1954	1955	1956	1957	4-Year average
Streambank wheatgrass	Alternate rows with alfalfa	2.60	3.60	1.03	.34	1.89
	Mixture with alfalfa	2.68	3.04	.82	.18	1.68
	Grass alone and fertilizer	2.67	1.80	.34	.15	1.24
Crested wheatgrass	Alternate rows with alfalfa	3.12	3.48	.78	.64	2.00
	Mixture with alfalfa	2.95	3.57	.71	.21	1.86
	Grass alone and fertilizer	3.37	2.16	.49	.24	1.56
Intermediate wheatgrass	Alternate rows with alfalfa	3.60	3.86	1.33	.58	2.34
	Mixture with alfalfa	3.37	3.35	.83	.25	1.95
	Grass alone and fertilizer	4.07	2.73	.55	.26	1.90
Year mean		3.16	3.06	.77	.32	

ANALYSIS SUMMARY

Source of variation	D.F.	Mean square
Grass species	2	2.55*
Replicates	3	.37
Grass species × replicates (Error A)	6	.25
Seeding methods	2	3.15**
Seeding methods × grass species	4	.20
Seeding methods × grass spp. × replicates (Error B)	18	.37
Years	3	80.60**
Years × grass species	6	.55
Years × seeding methods	6	1.77*
Remainder (Error C)	93	.48

*Significant at 5% level
**Significant at 1% level

The test was harvested for hay each year commencing in 1954. In 1954 and 1955 two cuttings were taken, while in 1956 and in 1957 only one cutting was made. In the pure grass sub-plots and in the mixture sub-plots three rows were cut for hay. In the alternate row sub-plots two rows of grass and two of alfalfa were cut separately. Samples from each sub-plot were retained for dry matter determinations. The samples from the mixture sub-plots were hand-separated into grass and alfalfa portions before drying.

RESULTS

The yield data, and variance analysis summary, are presented in Table 3. Yield differences between grasses were significant, while the yield differences due to seeding methods and to years were highly significant. The only significant yield interaction was between years and seeding methods. This was undoubtedly due to the 1954 yields from the fertilized pure grass stands which were as great or greater than the forage yields from sub-plots containing alfalfa.

The yield data, according to the performance of grass species and seeding methods, are presented in Tables 4 and 5 respectively. A substantial forage yield advantage from alternate-row stands compared to mixture stands is evident for each year except (see Table 3) with streambank wheatgrass in 1954 and crested wheatgrass in 1955. This average yearly increase

TABLE 4.—FORAGE YIELD ACCORDING TO GRASS SPECIES BY YEARS

Grass species	Dry matter yield—Tons per acre				
	1954	1955	1956	1957	4-year average
Streambank wheatgrass	2.65	2.81	.79	.22	1.61
Crested wheatgrass	3.15	3.07	.66	.36	1.81
Intermediate wheatgrass	3.68	3.31	.90	.36	2.07
L.S.D. (P=.05)	.55	.17	.22	.09	.24

TABLE 5.—FORAGE YIELD ACCORDING TO SEEDING METHODS BY YEARS

Seeding method	Dry matter yield—Tons per acre				
	1954	1955	1956	1957	4-year average
Alternate row: grass−alfalfa	3.11	3.65	1.05	.52	2.08
Mixture: grass+alfalfa	3.00	3.32	.79	.22	1.83
Grass alone and fertilizer	3.37	2.23	.46	.22	1.57
L.D.S. (P=.05)-	.14	.30	.04	.03	.24

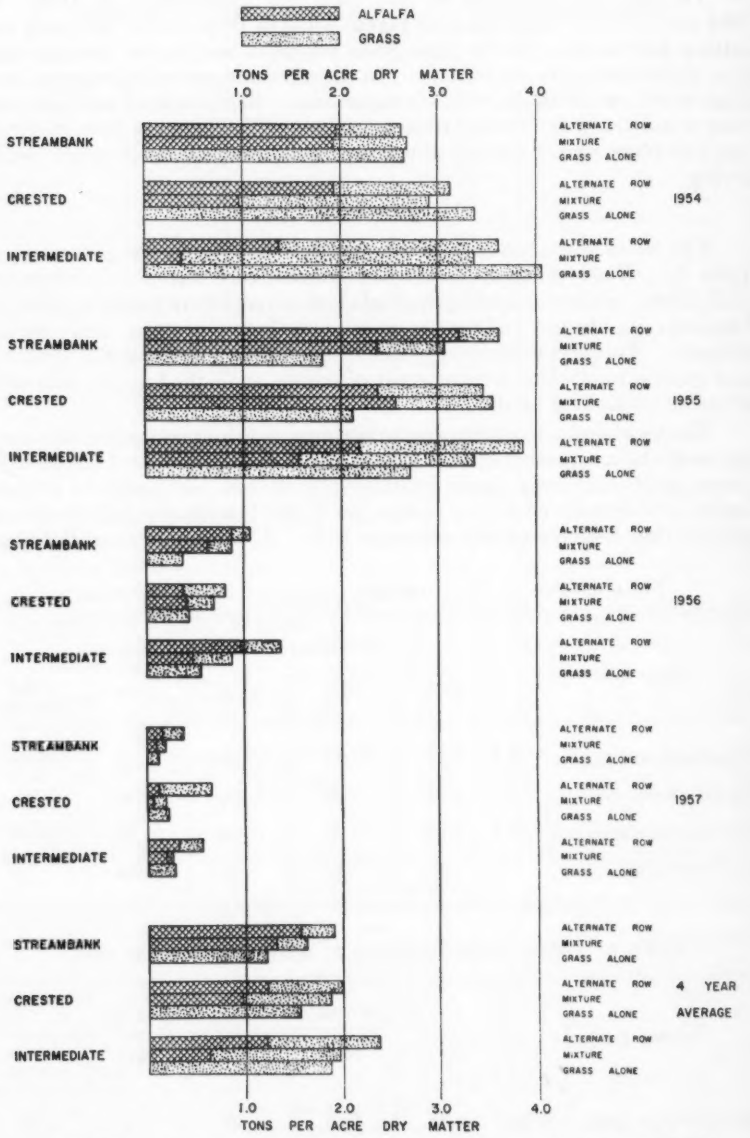

FIGURE 2. Comparative forage yields for each crop, showing grass and alfalfa components according to species, seeding methods, and years.

in forage yield from alternate rows over mixture rows was 4 per cent in 1954; 10 per cent in 1955; 33 per cent in 1956, and 137 per cent in 1957. The 4-year average yield showed that alternate-row stands of grass and

TABLE 6.—INFLUENCE OF SEEDING METHOD ON YIELD OF GRASS PORTION—4-YEAR AVERAGE

Grass species	D.M. yield Tons per acre			Relative yield Performance by %		
	Alternate row	Mixture	Alone	Alternate row	Mixture	Alone
Streambank wheatgrass	.35	.40	1.24	100	100	100
Crested wheatgrass	.80	.88	1.56	229	220	126
Intermediate wheatgrass	1.12	1.07	1.90	320	268	153

TABLE 7.—PERCENTAGE BASAL GROUND COVER OF THE GRASSES AND ALFALFA IN 1957 (Mean value of 4 Replicates)

Grass species	Alternate row		Mixture		Grass alone
	Grass	Alfalfa	Grass	Alfalfa	
Streambank wheatgrass	17.77	3.89	14.72	3.55	30.98
Crested wheatgrass	16.54	3.61	20.14	1.88	22.66
Intermediate wheatgrass	12.07	4.59	12.77	2.49	23.75
Mean	15.44	4.03	15.88	2.64	25.80

alfalfa provided 13 per cent more hay than did mixed stands. It is important to note that in dry years (1956 and 1957, Table 2) the advantage of alternate-row seeding was very marked. May precipitation, so important for perennial forage growth, was very low during these 2 years. Grass alone, even though fertilized, yielded less than when seeded with alfalfa in all years except the first.

The yield data identifying the grass and the alfalfa portions are presented graphically in Figure 2. With two exceptions, the total forage yields in each year from alternate-row stands were greater than yields from mixed stands. This performance was true for each grass species. It will be noted, however, that the increased yields from alternate rows were the result of a greater alfalfa yield than occurred in mixed stands. The greater yield of alfalfa in alternate rows usually was associated with a decrease in the yield of grass as compared to the yield of grass in mixed stands.

The 4-year average yield data, of the grass portion only, are presented in Table 6 by species and seeding methods. For each seeding method the order of grass species yield performance was the same but the magnitude of the difference between the species yield was influenced greatly by the seeding method. By giving streambank wheatgrass a percentage yield value of 100 for each seeding method, the relative yield of each of the other species was computed and recorded in the last three columns of Table 6. Thus the difference between streambank wheatgrass, the low yielding species,

and intermediate wheatgrass, the high yielding species, was 53 per cent when they were grown in pure stands, 168 per cent when grown in mixture with alfalfa, and 220 per cent when grown in alternate rows with alfalfa.

The percentage basal ground cover for each plot within each replicate was determined by the point quadrat method in 1957. The mean data presented in Table 7 show that there was more alfalfa in the alternate row sub-plots than in those where the grass and alfalfa were growing in mixture. This was particularly so where crested wheatgrass and intermediate wheatgrass occurred, and was probably caused by greater shade from these two grasses on the alfalfa during the seedling year when grass and alfalfa occurred in mixed rows. Crested wheatgrass in mixture with alfalfa was the most competitive grass species, as indicated by the 20.14 per cent ground cover which was nearly as great as the 22.66 per cent ground cover of crested wheatgrass by itself.

The strong sod-forming quality of streambank wheatgrass when seeded by itself is also apparent by the 30.98 per cent ground cover as compared to the ground cover for the other grasses by themselves.

DISCUSSION

The results from the experiment indicate that the three grasses—crested wheatgrass, intermediate wheatgrass, and streambank wheatgrass—although performing in relatively the same order, displayed changing degrees of response when seeded in different ways with alfalfa. This influence of seeding method on species response supports the argument offered by others (5, 10, 15, 17), that grasses should be tested and compared under conditions in which they are most likely to be used.

Although total forage yields for grasses and alfalfa in alternately seeded rows were greater than yields for grasses and alfalfa grown in mixture, the influence of seeding method on percentage composition of the forage may be the more important factor. The amount of alfalfa, in both ground cover and yield, was greater in alternate-row stands than in mixed stands, indicating that alfalfa would likely remain in the stand for a greater number of years when seeded in this manner and thereby extend the productive life of the hay field.

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THE USE OF NITROGENOUS FERTILIZER IN THE PRODUCTION OF GRASSLAND HERBAGE[1]

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ABSTRACT

Ammonium nitrate and calcium cyanamide were applied to an orchard grass, ladino clover sward over a 3-year period. Rates to give 0, 50, 100 and 150 lb. of nitrogen per acre were applied in two ways: single spring applications, and split applications, one-half in the spring and one-half immediately following an early first cutting.

Ammonium nitrate proved to be the more effective source of nitrogen whether measured by yield, crude protein of the herbage or net recovery of nitrogen.

Single spring applications of nitrogenous fertilizer gave greater seasonal total yields of dry matter and crude protein and more efficient net recovery of nitrogen than did split applications.

Where no nitrogen was applied there was an increase in the clover content of the sward, total yield of dry matter and the total yield of crude protein during the 3 years of the test. However, the greatest seasonal total from these plots did not equal that obtained from the highest rate of application of fertilizer nitrogen. The highest of the rates almost completely suppressed clover development but gave the greatest yields of dry matter and crude protein and the most efficient net recoveries of nitrogen.

INTRODUCTION

The paramount importance of nitrogen in the production of grassland herbage has been noted by Holmes (3, 4, 5, 6) working in England and by Sprague and Garber (7) and Robinson and Sprague (8) in the United States.

Holmes (6) and Robinson and Sprague (8) concluded that continuous production of herbage could be obtained by regular applications of nitrogen throughout the season. Walker and co-workers (9, 10, 11) found that, provided sufficient mineral nutrients were available, satisfactory yields of herbage could be obtained by nitrogenous fertilization.

Sprague and Garber (7) and Walker (10, 11) both reported suppression of clover under heavy nitrogen fertilization. Walker (11) in a discussion of this aspect suggested that, under a system of management where the first cutting of herbage is removed early enough, clover suppression would not be a serious problem.

In general, the literature cited above shows that heavy yields of grassland herbage can be obtained by generous applications of nitrogenous fertilizer.

The purpose of the investigation reported here was to examine, under Nova Scotia conditions, the comparative effects of two sources of nitrogen at different rates and times of application on the total yield, seasonal distribution of the yield and the protein content of the herbage.

MATERIALS AND METHODS

The area used consisted of a mixed orchard grass, ladino clover sward seeded 2 years previous to the beginning of the experiment.

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Four rates of nitrogenous fertilizer were used to give 0, 50, 100 and 150 lb. of actual nitrogen per acre. In one series of plots all the nitrogenous fertilizer was put on in a single application in the spring. In a second series, one-half the nitrogen was put on in the spring and one-half immediately following the first cutting of herbage. Each series was repeated, including the zero rates, with both ammonium nitrate and calcium cyanamide. The resulting 16 plots were replicated four times in randomized blocks.

The plots were 8 feet wide by 50 feet long. All fertilizer was applied by means of a previously calibrated fertilizer distributor drawn by a light tractor.

In addition to nitrogenous fertilizer, an over-all application of 500 lb. of 20 per cent superphosphate and 100 lb. per acre of 60 per cent muriate of potash was made each year in the spring. In the first year, 25 lb. of borax per acre was applied over the whole area.

The herbage was harvested by clipping one 30-inch strip with a sickle bar mower over the length of the plot. Following this the whole area was mowed and all herbage removed. Green weight of the 30-inch strip was recorded and a sample of approximately 1 lb. taken for dry matter determination. These samples were subsequently ground and used for nitrogen determination. In order to assess the clover population of the sward a clover-grass separation of the samples from two of the four replications was made prior to dry matter determination, the clover and grass portions of the sample being dried and weighed separately. Following drying the samples were ground in a Wiley mill and stored in screw-top glass bottles prior to nitrogen analysis.

Total nitrogen was determined by the A.O.A.C. micro-distillation technique (2) after Kjeldahl digestion of the sample with a mixture of sulphuric and phosphoric acids using selenium and copper salts as catalysts (12). Crude protein content was calculated in the usual way by multiplying total nitrogen by the factor 6.25.

The herbage was cut three times in the first 2 years of the test but in the last year only two cuts were made.

Growing conditions in 1956 were not nearly as good as in the first 2 years of the test. July and August temperatures were below normal and, in addition, August precipitation was much below the previous 2 years. However, the reason for only two cuts in 1956 was not entirely due to the weather. An error in judgement as to the best time to make the second cutting resulted in its being taken too late. Consequently there was insufficient recovery of the sward for a third cutting in that year. First cuts were taken on June 6, 1954, June 9, 1955, and June 14, 1956.

RESULTS

An analysis of variance was made of all the data for yield and crude protein content. In general, the 3-year averages adequately represent the data obtained over the period of this test; therefore, except in instances where annual trends need to be emphasized, only 3-year averages are presented.

TABLE 1.—YIELD OF HERBAGE AT THREE CUTTING DATES AND FOUR LEVELS OF NITROGEN FERTILIZATION. 3-YEAR AVERAGE DRY MATTER PER ACRE

Lb. nitrogen per acre	First cut	Second cut	Third cut	Total, all cuts
	lb.	lb.	lb.	lb.
0	3405	1424	524	5353
50	3563	1402	350	5315
100	4397	1626	323	6345
150	4604	1961	453	7018
L.S.D. .05	355	127	95	423

Increased applications of nitrogen produced increased yields of dry matter (Table 1) but it is quite apparent that much the greater part of the increase occurred at the time of the first cutting. Furthermore, ammonium nitrate gave a significantly greater average yield of herbage than calcium cyanamide (Table 2). There was, however, a significant interaction between quantity and source of nitrogen and it is quite evident that the yield difference in favour of ammonium nitrate increased with the rate of nitrogenous fertilization. On the other hand, the difference between ammonium nitrate and calcium cyanamide decreased from 1914 to 447 lb. of dry matter per acre between the first and last years of the test, although the mean yield from ammonium nitrate remained fairly constant.

Splitting the application of nitrogenous fertilizer gave a significantly lower average yield of dry matter than single spring applications (Table 3). In 2 of the 3 years of the test the second cutting yield was significantly increased by the split application, although this increase did not compensate for the reduction in yield in the first cutting. Ammonium nitrate was somewhat more effective in this respect than calcium cyanamide. The average increase in second-cut yield, due to splitting the ammonium nitrate application, was 278 lb. of dry matter per acre, while that for calcium cyanamide was 91 lb.

The plots receiving no nitrogenous fertilizer produced a much greater increase in yield of dry matter, between the first and last year of the test, than did the plots receiving nitrogen (Table 4). In fact, this improvement in yield between the first and last year became progressively less as the nitrogenous fertilizer was increased. The relative change between the second and last year is even more marked and in the last year the zero nitrogen plots outyielded the nitrogen fertilized plots, although the difference approached significance only in the case of the 50 lb. per acre rate.

The effect of quantity of nitrogen on crude protein content of the herbage was significant at all cuttings (Table 5). However, it was only at the first cuttings that protein content increased with nitrogen applications. At the other cuts the zero nitrogen treatments had the higher protein content. Total crude protein harvested increased with increased application of nitrogen although there was no difference between the zero and the 50-lb. per acre rate. Net recovery of nitrogen also increased markedly with increased application of nitrogen (Table 5).

TABLE 2.—YIELD OF HERBAGE WITH TWO SOURCES OF NITROGEN AT THREE RATES. 3-YEAR AVERAGE, DRY MATTER PER ACRE

Lb. nitrogen per acre	First cut		Second cut		Third cut		Total, all cuts	
	Ammonium nitrate	Calcium cyanamide	Ammonium nitrate	Calcium cyanamide	Ammonium nitrate	Calcium cyanamide	Ammonium nitrate	Calcium cyanamide
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
50	3843	3283	1383	1421	295	405	5521	5109
100	4906	3887	1664	1588	238	407	6808	5882
150	5365	3843	2138	1784	445	459	7948	6086
Mean	4705	3671	1728	1598	326	423	6759	5692
L.D.S. .05 rates of nitrogen	502	502	180	180	135	135	598	598
L.S.D. .05 means of sources of nitrogen	290		104		78		345	

TABLE 3.—YIELD OF HERBAGE WITH TWO METHODS OF APPLICATION OF NITROGEN AT THREE RATES. 3-YEAR AVERAGE, DRY MATTER PER ACRE

Lb. nitrogen per acre	First cut		Second cut		Third cut		Total, all cuts	
	Single application	Split application	Single application	Split application	Single application	Split application	Single application	Split application
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
50	3829	3297	1365	1439	376	324	5570	5060
100	4813	3980	1539	1713	328	317	6680	6010
150	4880	4328	1810	2112	512	393	7202	6833
Mean	4507	3868	1571	1755	406	345	6484	5968
L.S.D. .05 rates of nitrogen	502	502	180	180	135	180	598	598
L.S.D. .05 means of times of application	290		104		78		345	

TABLE 4.—YIELD OF HERBAGE AT FOUR RATES OF APPLICATION OF NITROGEN FOR 3 YEARS. DRY MATTER PER ACRE

Year	Lb. nitrogen per acre			
	0	50	100	150
	lb.	lb.	lb.	lb.
First	3405	3933	5294	5976
Second	6338	6160	7443	8298
Third	6317	5585	6018	6258

L.S.D. .05 between quantities of nitrogen within years—736 lb. per acre

TABLE 5.—CRUDE PROTEIN AS PER CENT OF DRY MATTER, TOTAL CRUDE PROTEIN PER ACRE AND NET RECOVERY OF NITROGEN AT FOUR RATES OF APPLICATION OF NITROGENOUS FERTILIZER. AVERAGE OF 3 YEARS

Lb. nitrogen per acre	First cut	Second cut	Third cut	Total all cuts	Net recovery of nitrogen
	%	%	%	lb.	%
0	13.22	13.83	17.16	706	
50	14.06	12.77	14.83	699	−2.2
100	14.84	12.43	13.65	815	25.5
150	15.81	13.19	13.65	1013	32.7
L.S.D. .05	1.13	.75	.81	85	

The effect of source of nitrogen and time of application of nitrogen on crude protein content of the herbage (Table 6) was significant at the first cut only. There ammonium nitrate gave a significantly higher crude protein content than calcium cyanamide and the single spring application a higher crude protein content than the split application.

Ammonium nitrate gave a significantly higher total yield of crude protein than did calcium cyanamide and the single spring application of nitrogen gave a significantly higher total yield than the split application (Table 6). This superiority of ammonium nitrate and of the single spring application is mainly due to the large yield of high crude protein herbage obtained at the first cut.

Ammonium nitrate gave a much higher net recovery of nitrogen than did calcium cyanamide, and the single spring application much higher than the split nitrogen application.

In the no-nitrogen and 50-lb. rate plots there was a marked increase in the clover content of the herbage over the 3 years of the test (Table 7). The same tendency was evident at the 100-lb. rate where calcium cyanamide was the source of nitrogen. At the highest rate of nitrogen fertilization clover was very much reduced, although the data indicate that there was more clover development when calcium cyanamide was the source of nitrogen.

TABLE 6.—CRUDE PROTEIN AS PER CENT OF DRY MATTER, TOTAL CRUDE PROTEIN PER ACRE AND NET RECOVERY OF NITROGEN WITH TWO SOURCES AND TWO METHODS OF APPLICATION OF NITROGENOUS FERTILIZER. AVERAGE OF 3 YEARS

	Source of nitrogen			Method of application of nitrogen		
	Ammonium nitrate	Calcium cyanamide	L.S.D. .05	Single application	Split application	L.S.D. .05
Crude protein first cut, %	16.05	14.40	.65	17.00	13.76	.65
Crude protein per acre total, 3 cuts, lb.	957	761	.69	960	758	.69
Net recovery of nitrogen, %	31.0	8.6		38.0	.7	

TABLE 7.—CLOVER CONTENT AS PER CENT OF DRY MATTER HARVESTED AT THE LAST CUT WITH TWO SOURCES OF NITROGEN AT FOUR RATES OF APPLICATION

Lb. nitrogen per acre	First year		Second year		Third year	
	Ammonium nitrate	Calcium cyanamide	Ammonium nitrate	Calcium cyanamide	Ammonium nitrate	Calcium cyanamide
	%		%		%	
0	20.6		22.7		33.5	
50	3.9	8.6	26.2	22.9	29.7	21.0
100	0.8	6.6	7.8	13.4	5.7	21.7
150	0.0	2.5	3.8	6.3	0.0	6.9

DISCUSSION

In assessing the effect of the nitrogenous fertilization yields of dry matter and crude protein and clover population must be considered.

It is clear that yields of dry matter and crude protein and net recovery of nitrogen rose markedly as the rate of application of nitrogen was increased. There was, however, a considerable narrowing of the difference in yield of dry matter between the first and last years of the test. The latter effect was due to an improvement in the yield of the zero nitrogen plots which in turn was clearly caused by an increase in the clover population of these plots. It would appear that if fertilizer nitrogen is to be used it must be applied in sufficient quantity that its effect on the yield of grass is large enough to offset the deleterious effect it has on clover development. From a strictly practical standpoint it would be necessary to decide whether the high rate of application would pay for itself in extra herbage and crude protein. The data from this test indicate that no nitrogen was better than the 50-lb. rate.

It seems clear that calcium cyanamide was less detrimental to clover than ammonium nitrate although the added clover produced did not compensate for the greater yield of dry matter and crude protein obtained when ammonium nitrate was the source of nitrogen.

Delaying one-half the nitrogen fertilizer application until after the first cutting did not at any time produce an increase in yield to compensate for that sacrificed at the first cutting, although with ammonium nitrate splitting the nitrogenous fertilizer did produce sizable increases in second-cut yield. Since extra herbage is frequently required for grazing, at the time the second cut was taken, the use of a split application might be desirable in spite of the resulting reduction in total herbage yield. In addition to the reduction in yield of dry matter splitting the nitrogenous fertilizer lowered the crude protein content of the first cut, the total yield of crude protein and the efficiency of nitrogen recovery.

Walker (11) suggested that where the first cut was removed early enough clover suppression as a result of nitrogenous fertilization would not be serious. In this experiment the first cuttings were made early in the season and it is doubtful if under local climatic conditions earlier cutting would be possible. Suppression of the clover due to shading by the grass was, therefore, at a minimum in so far as it could be influenced by early removal of the herbage by cutting. In spite of this, clover was practically eliminated at the highest level of nitrogenous fertilization. It seems likely, therefore, with a mixed grass clover sward, if full advantage is to be had from the legumes on the one hand or nitrogenous fertilizer on the other, the choice must be between no fertilizer nitrogen or relatively large amounts.

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NOTE ON LEAF RUST REACTION OF CHINESE SPRING (THATCHER) SUBSTITUTION LINES[1]

The widely grown hard red spring wheat variety, Thatcher, is susceptible to all races of leaf rust, *Puccinia triticina* Eriks., commonly found in western Canada. Chinese Spring, the variety in which the substitution lines have been developed by E. R. Sears, has a high degree of mature plant resistance to leaf rust, though it is susceptible to stem rust.

Chinese Spring lines having chromosomes from a number of stem rust resistant donor varieties have been successfully used by Sears and Rodenhiser (2) and Sears *et al.* (3) to identify the chromosomes that carry genes for resistance to stem rust. In such studies the susceptible genetic background of Chinese Spring reveals the critical chromosome of the donor variety by the resistant reaction caused by the gene or genes introduced by such a chromosome. With leaf rust reaction, when Chinese Spring lines with substituted chromosomes are used, the situation is different. The genetic background of the recipient variety now is resistant and the genetic differences of the two varieties in question can only be revealed if there is at least a recognizable degree of susceptibility of the critical substitution line or lines.

TABLE 1.—PERCENTAGE LEAF RUST ON LEAVES OF CHINESE SPRING (THATCHER) SUBSTITUTION LINES, WINNIPEG, 1954, 1955

Thatcher Chromosome tested	1954 results (mean 6 replicates)	1955 results (mean 2 replicates)	Mean 1954-55
I	Trace	Trace	Trace
II	Trace	Trace	Trace
III	Trace	Trace	Trace
IV	Trace	Trace	Trace
V	Trace	Trace	Trace
VI	Trace	Trace	Trace
VII	Trace	Trace	Trace
VIII	Trace	Trace	Trace
IX	23.4	13.0	18.2
X	1.5	20.0	11.2
XI	Trace	Trace	Trace
XII	1.0	28.0	14.5
XIII	Trace	Trace	Trace
XIV	Trace	Trace	Trace
XV	Trace	Trace	Trace
XVI	Trace	Trace	Trace
XVIII	Trace	Trace	Trace
XIX	Trace	Trace	Trace
XX	Trace	Trace	Trace
XXI	26.3	25.0	25.6
Thatcher	78.0	90.0	84.0
Chinese Spring	Trace	Trace	Trace

[1] Contribution from the Department of Plant Science, University of Alberta, Edmonton, Alta., and the Cereal Breeding Laboratory, Canada Department of Agriculture, Winnipeg, Man.

The Chinese Spring (Thatcher) substitution lines were grown in the field rust nursery of the Cereal Breeding Laboratory at Winnipeg, Manitoba, during the summers of 1954 and 1955. The prevalent leaf rust races in both years were 5a, 15a, and 126a. Heavy infection of leaf rust on susceptible varieties occurred in both years, but in 1954 the epidemic was late, and, because of severe stem rust infection on the leaves, there was some difficulty in obtaining accurate leaf rust readings. Readings of percentage leaf rust were based on the scale of Peterson *et al.* (1). Readings below 1 per cent on this scale are recorded as "trace".

As shown in Table 1, four substitution lines carried more leaf rust than Chinese Spring in both 1954 and 1955, indicating that Chinese Spring and Thatcher differ by genes on chromosomes IX, X, XII, and XXI controlling leaf rust reaction.

The inconsistency of lines X and XII in degree of infection in the two years is probably not due to differences in physiologic races of leaf rust since the prevalent races were the same in both years. The low infection recorded for these two lines in 1954 may have resulted from the later outbreak of the epidemic in that year allowing more time for the more effective genes to bring about mature plant resistance. The genes for leaf rust resistance in chromosomes IX and XXI appear to have been more effective in this regard than the genes on chromosomes X and XII. This situation merits further investigation.

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NOTE ON THE PLACE OF A LOGARITHMIC SPRAYER IN TESTING HERBICIDES FOR WEED CONTROL[1]

The logarithmic sprayer is so designed that the rate of application of herbicide decreases in an exponential manner along the length of the plot from the initial rate of application selected (3). The author has used the Chesterford Logarithmic Spraying Machine for one summer. The purpose of the present Note is to assess the machine for its practicability in weed-control experiments.

The sprayer was mounted on a jeep and driven from the power take-off. The pump delivered 34 gallons per acre at a speed of 4 m.p.h., and the rate of application of the herbicide decreased continuously along the length of the plot, so that the rate was halved at 21-foot intervals. The principles involved which permit this decreasing application have already been described (1).

This method has the advantage of applying a complete spectrum of rates within one plot. Considerable time is saved, in that only one solution strength must be mixed for each spectrum. This method of spraying is particularly advantageous for screening new herbicides, to ascertain the range of concentration for crop tolerance and weed kill. A high initial rate of application can be selected in the first trials, and when an appropriate range of solution strength has been selected, refinement may be obtained by decreasing the initial rate. Establishment of recommended rates can thus be obtained. For example, butyl ester of 2,4-Dichlorophenoxyacetic acid, applied at an initial rate of 25 lb. to absinthe (*Artemisia absinthium*), provided 100 per cent kill of top growth at rates ranging from 25 to 13.4 lb. acid equivalent per acre (Table 1). Further tests are necessary, however, to establish effective minimum rates under various conditions.

A portion of each plot may be used as a check if the design is so arranged that the light application end of one plot is adjacent to the heavy application end of another. Conventional check plots, however, are also necessary.

The logarithmic sprayer lends confidence to results obtained from pre-emergence applications of chemicals to various weed species. Where one must rely entirely on check plots, there is the tendency for the worker to ponder whether the apparent inhibition of germination of the weed seeds occurred as a result of the treatment, or whether it was caused by dormancy of seed, or by unfavourable growing conditions. The use of the logarithmic sprayer assists in quelling some of these doubts. Weed growth may be totally inhibited at the high rate of application, grade off into a few

TABLE 1.—THE AMOUNT OF THE BUTYL ESTER OF 2,4-D REQUIRED (OZ. PER ACRE) TO PROVIDE VARIOUS PERCENTAGES OF TOP KILL OF THREE WEED SPECIES

Weed species	Percentage kill			
	100	90	80	70
Artemisia absinthium	13.4	7.8	—	—
Salsola pestifer	—	44	23	—
Polygonum convolvulus	60	35	30	24

[1] Contribution from the Department of Plant Ecology, University of Saskatchewan, Saskatoon, Sask., with financial assistance from United Grain Growers Ltd.

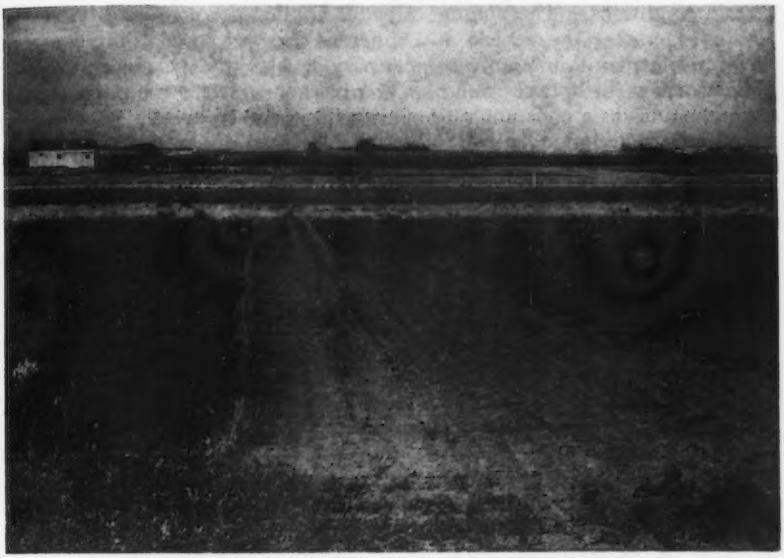

FIGURE 1. Pre-emergence application of trichlorobenzoic acid to broad-leaved and grassy weeds, May 9, 1957. The herbicide was applied with a Chesterford Logarithmic Spraying Machine at a rate which decreased from 24 lb. (*foreground*) to 0.5 lb. active ingredient per acre.

unthrifty shoots at a lighter rate, and become more vigorous as rates decrease further, thus providing strong evidence that the results are caused by the treatment. Typical results are shown for pre-emergence application of trichlorobenzoic acid applied at rates of 24 to 0.5 lb. per acre (Figure 1).

The sprayer has considerable versatility, in that it may be used to deliver two herbicides simultaneously, with the heavy rate of one herbicide, and the light rate of another applied to the same end of the plot (3). The sprayer also permits variation in plot size. The length of plot required depends upon the concentration of the initial rate and the range of the rate spectrum required for the experiment in question. For example, in a plot 135 feet long, using an initial rate of 8 lb. per acre, the rate at the end of the plot would be 2.4 oz. This distance may be shortened to include only the critical range of rates for the species in question. The boom of the sprayer is in three sections, so that it may be folded for transportation. The sprayer also may be operated in this position, so that the width of the plot may be either 5 feet (with the booms folded) or 15 feet with the booms extended. The use of the narrow plot, however, has the disadvantage of increasing by three times the amount of water applied per acre. This may be considered an advantage in experiments which involve the effectiveness of the herbicide applied with different volumes of water. Two volumes may be applied simultaneously by spraying with one section of the boom folded. This effectively doubles both the rate of herbicide and the volume of material applied. Dorschner and Buchholtz (2) have shown that the volume of water applied influenced the effectiveness of the herbicide under

certain conditions. The volume of water applied per acre may be varied also by operating the vehicle at a different forward speed (different gear ratio) while maintaining the same r.p.m. level with the power take-off (which drives the sprayer pump). A governor which is mounted on the engine maintains a constant rate of travel, even over rough terrain. The jeep-mounted unit is particularly adaptable for spraying natural infestations which may be located several miles from the base of operations. For this reason, it may be useful also in other phases of agriculture, for example entomology or plant pathology.

A disadvantage of the Chesterford Logarithmic Spraying Machine is that it delivers more water per acre than is generally applied by farmers in Western Canada. Admittedly the volume may be decreased by travelling at a higher speed; but often the roughness of the terrain discourages the higher rate of travel. Another disadvantage is apparent when one tests the effects of herbicides on crop yields. The spectrum effect necessitates a yield test for a range of rates rather than for a single rate of application.

A higher proportion of weeds consistently survived in the wheel-tracks of the jeep than elsewhere in the plot. The crushing effect of the wheels travelling over the plants may have decreased the amount of leaf surface available for contact with the herbicide, or the plants may have been covered with sufficient dust raised by the rubber lugs of the tires to decrease the efficiency of the herbicide.

Results obtained with the logarithmic sprayer indicated that the concentration of a herbicide must reach a specific level, or threshold, before the plant is adversely affected. This was shown in plots of leafy spurge (*Euphorbia esula*) and Russian thistle (*Salsola pestifer*). A detrimental effect could not be observed on Russian thistle with the application of the butyl ester of 2,4-D at a rate less than 4 oz. acid equivalent per acre. At this rate, curling of the tips occurred so abruptly that a straight line was visible across the plot. A similar phenomenon occurred when the same herbicide was applied to leafy spurge. Although the rate of application of the herbicide decreased continuously along the plot, the top growth of the weed changed abruptly in appearance so that the plot could be divided into five definite sections. The plants were brown and desiccated at rates from 103.5 to 42.1 oz. per acre, passing through three distinct zones to the fourth, where the shoots were green and curled at rates from 6.9 to 2.3 oz. per acre (Table 2). The transition zones were very narrow between these sections, rarely exceeding 2 to 3 feet in width. This effect was no longer visible after a period of one month had elapsed.

TABLE 2.—THE EFFECT OF VARIOUS RATES OF THE BUTYL ESTER OF 2,4-D ON THE VEGETATIVE SHOOTS OF *E. esula*

Rate range (oz. per acre)	Colour and appearance of shoots
2.3– 6.9	Green, tips curled
6.9–16.0	Yellow, tips curled
16.0–21.6	Red, tips curled
21.6–42.1	Reddish brown
42.1–103.5	Brown and shrivelled

The inherent variability which occurs in biological material is well known. It is logical that different plants of the same species should exhibit various degrees of tolerance to herbicides. This was substantiated by results obtained with the logarithmic sprayer. For example, 60 oz. acid equivalent per acre of the butyl ester of 2,4-D killed 100 per cent of the wild buckwheat (*Polygonum convolvulus*) population, while at the rate of 24 oz. per acre 30 per cent of the plants survived (Table 1). Similarly, after three applications of amino triazole at 40 oz. active ingredient per acre, 5 per cent of the Russian thistle population survived, while 30 per cent of the plants survived three treatments of 22 oz. per acre. Similar results were obtained with perennial weeds. Trichlorobenzoic acid at 15.5 lb. per acre active ingredient provided 100 per cent kill of absinthe top-growth, while the 6.4-lb. rate killed 50 per cent. Similarly, 2,4,5-Trichlorophenoxyacetic acid at a rate of 5.1 lb. per acre killed 90 per cent of the weed, while 3 lb. per acre killed 80 per cent. At rates below the lethal dose, considerable variation occurred also in the degree of injury. Wild buckwheat plants which were almost completely undamaged were found growing adjacent to those which were severely affected at a range of application between 4 oz. and 14 oz. acid equivalent per acre. Satisfactory control was obtained with the 14-oz. rate. More than four times this amount was required to provide 100 per cent kill, and nearly twice as much to provide 70 per cent kill (Table 1).

The many advantages of the logarithmic sprayer readily recommend its use in experimental trials involving the applications of herbicides to weeds and crop plants. Information obtained suggests a partial answer to the question often posed by farmers, that certain weeds (i.e. wild buckwheat) are becoming more resistant to 2,4-D. The explanation given has been that the eradication of the more susceptible weed species by 2,4-D, and the consequent reduction in competition, may be contributing to the vigour of the weed. The above data suggest that, in eliminating the more susceptible individuals within a species, the herbicide is effectively selecting for the more resistant types. The fact that weed workers are concerned more about chemical weed control, rather than complete eradication, may be contributing to the eventual development of 2,4-D-resistant strains of weeds.

ACKNOWLEDGEMENT

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NOTE ON RESISTANCE OF SUNFLOWERS TO LEAF MOTTLE DISEASE

Wilt caused by species of *Verticillium* has been reported in over 120 plant species (3). Recent findings have shown that *V. albo-atrum* Reinke & Berth. is the causal organism of leaf mottle disease of sunflowers in Manitoba (2). The disease has been observed each year in Manitoba since 1948 and has caused serious economic losses to some growers, particularly in lighter soil areas, in three of the last four seasons. Sherbakoff (3) and Stevenson and Jones (4) reported that resistance to *Verticillium* or at least differences in susceptibility have been discovered in several plant species. This note reports evidence of resistance in the annual cultivated sunflower (*Helianthus annuus* L.).

Forty varieties and inbred lines of sunflowers were tested for resistance to *Verticillium* in 1957 by planting them in a test field which had grown sunflowers exhibiting severe leaf mottle disease in each of the three preceding years. Four replicates, of single-row plots, 20 feet long and 3 feet apart, were used with 40 seeds planted in each.

A record of the total plants in each plot and the number of these showing typical necrotic-chlorotic leaf mottle symptoms was made on August 7, at which time the majority of the lines were either in bloom or in the late bud stage. A second recording of the number of dead plants and the number of live plants with at least some green leaf tissue was made on September 11. On this second date most lines were approaching maturity. Leaf mottle symptoms were not positively identifiable on the dead plants. Consequently, only the infection on the live plants was recorded, but the

TABLE 1.—INCIDENCE OF LEAF MOTTLE DISEASE IN SUNFLOWER VARIETIES AND INBRED LINES UNDER NATURAL CONDITIONS AT WINKLER, MANITOBA, 1957

Variety	August 7		September 11	
	Total plants	% Diseased	Surviving plants	% Diseased
CM7	56	1.8	55	5.5
CM5	51	3.9	49	6.1
CM6	54	0.0	51	7.8
CM1	60	5.0	54	13.0
CM2	61	14.7	55	21.9
S-37-388	49	22.4	36	50.0
CM3	64	25.0	43	79.1
CM4	42	35.7	34	79.4
Sunrise	59	61.0	21	90.5
CM8	52	53.8	16	100.0
CM9	38	89.5	13	100.0

disease could have been the major reason for the dead plants. The features shown by the test are provided by the data in Table 1 which give a summary of the information obtained from 11 of the entries.

The lines CM4 and S-37-388 showed high infection. A cross between them produced the line CM3 with high infection, and the line CM2 with low infection. The symptoms on infected plants of CM2 were light in all four replicates. This line was among the earliest maturing in the test. Two other early lines, CM1 and CM5, also showed low infection. The line CM1 came from a double cross of S-37-388, CM4 and Sunrise, all with high infection, and a fourth line similar in appearance to Sunrise which was not included in the test. The line CM5 was selected from the variety Saratov.

The line CM9, which possesses the 953-102-1-1-22 rust resistant character (1), had high leaf mottle infection. The same was true of line CM8 which bears rust resistance from the 953-88 accession (1). In contrast, the two lines CM6 and CM7 which also contain rust resistance from the 953-88 source, had low leaf mottle infection. Further, the line CM7 was the latest in the test.

All lines which had low infection were either free of or only slightly affected by yellows, a disease prevalent on sunflowers in Manitoba in 1957 and believed to be caused by the aster yellows virus. The reverse did not hold. Among lines with high incidence of leaf mottle were some unaffected by yellows and others with up to 55 per cent of their plants showing the symptoms.

Percentage germination based on the plant count on August 7 was calculated for each line. Using these values for correlation with the percentage leaf mottle infection obtained on the same date an "r" value of −.408 was obtained, slightly in excess of the 1 per cent level of probability. This suggests that the infestation of *V. albo-atrum* in the soil was one factor contributing to the low plant stand.

Assuming that the low percentage of leaf mottle reported in some lines here is evidence of resistance to *Verticillium*, then the resistance has occurred in unrelated lines. It has appeared in both early and late lines, indicating that it is independent of time required to mature. The derivation of one line, CM2, of low infection, from a cross of two susceptible lines is an indication that the inheritance of the resistance is complex. Also it appears the leaf mottle resistance is not genetically linked either to the rust resistance from the 953-88 source or to the factors which govern the expression of symptoms due to the aster yellows virus.

Further investigation of the genetics of this resistance to leaf mottle and its incorporation into commercial varieties are contemplated. The latter is an important requirement because all sunflower varieties being grown at present in Manitoba are susceptible to the disease.

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NOTE ON A MACHINE FOR SUBSURFACE APPLICATION OF GRANULAR INSECTICIDES[1]

Several machines have been devised recently in the United States and Canada to apply granular insecticides to the surface of the soil or to the above-ground parts of plants for control of various insects. However, against root maggots attacking rutabagas in Prince Edward Island, where rutabagas are sown in ridges, surface applications are ineffective since the insecticide is moved away from the plants during cultivation and thinning; the best control has been obtained by placing a 5-inch band of insecticide 1½ inches below the seed in ridges 4 to 6 inches high[2]. With hand applicators it is difficult to obtain an even distribution of insecticide at the proper level in the soil and hand applications are impractical for large acreages. The machine described herein was devised to overcome these difficulties. This machine, which combines a ridger, applicator, hiller, and roller in one implement, gives precise applications at the desired level in the soil and saves much time and labour. It has been used successfully in Prince Edward Island to apply insecticides for control of root maggots in rutabagas and it may also be used against other soil insects attacking row crops.

CONSTRUCTION

The main details of the construction of the machine are shown in Figures 1 and 2. Those of the applicator are as follows: The insecticide hopper (Figure 2, B) is made of 18-gauge galvanized steel and the bottom fits the top of the feed mechanism. The feed mechanism is that of a fluted-feed grain drill, the direction of rotation of the feed cylinder being reversed and a piece of 12-gauge galvanized steel being connected to the casing and passing over the top of the feed cylinder to allow the granules to be metred evenly over the top of the cylinder.

The adjustable shoe (Figures 1, B; 2, C), which levels the soil at the desired height and spreads out the band of insecticide to the desired width, is made of ⅛-inch steel plate, the upright brace (E2) of inch diameter cold rolled steel, and the protective housing (E3) of 20-gauge galvanized steel connected to the flanges of the shoe with stove bolts. The blade (E1) is sharpened to a knifelike edge to enable it to cut through grass roots and other organic material in the soil. About 15 pieces of ¼-inch diameter cold rolled steel, 1 inch long, are welded to the face of the adjustable shoe to ensure an even distribution of the insecticide as it passes over the face of the shoe.

Except for the adjustable shoe, which requires forging, all parts of the machine and the materials used for the framework are readily available from most of the larger machine companies and blacksmith shops. At local retail prices, the total cost of the parts and materials for a two-row machine is approximately $160, about 20 hours' labour being required for its construction and assembly.

Blueprints showing details of construction of all parts of the machine may be obtained from the author.

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[1]Contribution No. 3768, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2]Read, D. C., and F. M. Cannon. Control of root maggots in rutabagas (Swede turnips) in Prince Edward Island. *In preparation.*

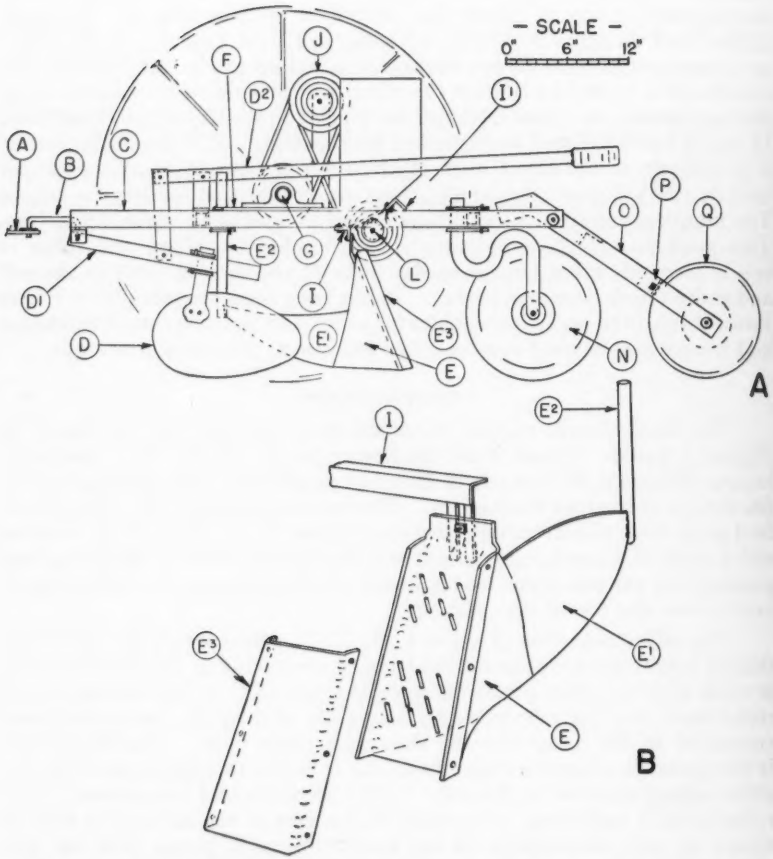

FIGURE 1. A, Scale diagram of a lateral view of one unit of the machine showing the various parts assembled for operation, the parts being labelled as follows: A, drawbar; B, connecting link; C, main frame; D, standard mouldboard hiller; D1, supporting framework for front hillers; D2, lever for raising front hillers; E, adjustable shoe; F, supporting brace for main-drive axle (G); H, steel wheel; I and I1, cross-member supports for insecticide hopper, feed mechanism, and back of adjustable shoe; J, brace support for idler pulleys; L, axle for feed mechanism; M, brace and brass bushing to support outer end of axle (L); N, standard 13-inch disk hiller with offset shank; O, supporting framework for concave rollers; P, flat-iron scraper; Q, standard concave roller from a two-row rutabaga seeder.

B. Details of construction of the adjustable shoe.

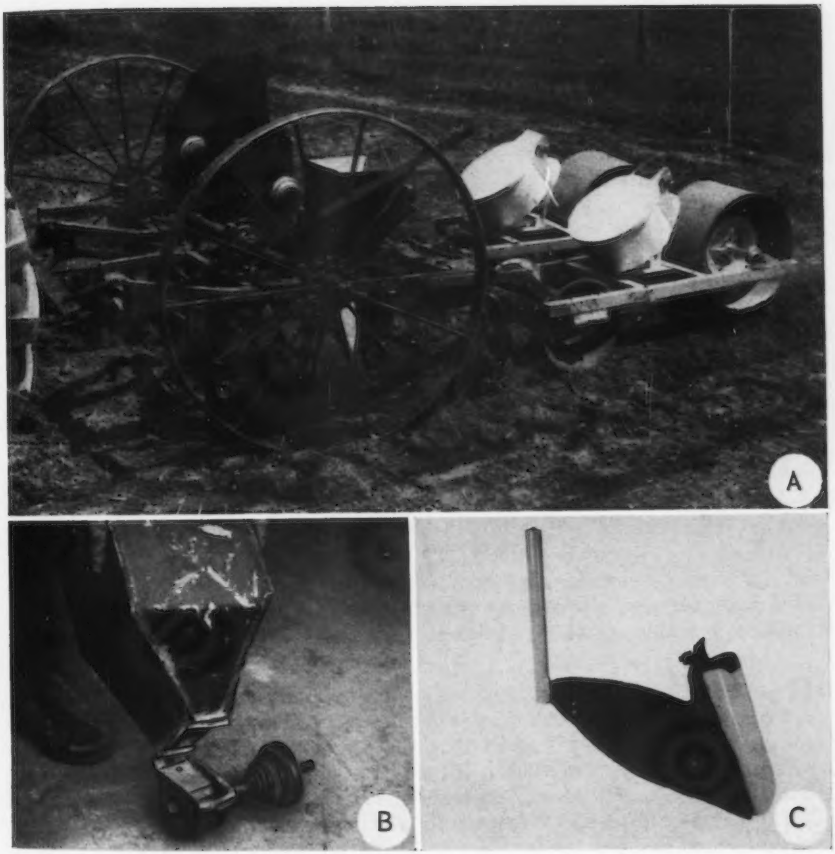

FIGURE 2. A, View of the machine in operation with a two-row rutabaga seeder connected at the back. B, Insecticide hopper and fluted feed mechanism. C, Adjustable shoe.

OPERATION

When the insecticide is to be placed in the soil above ground level, as in the control of root maggots in rutabagas grown in ridges, the front hillers (D) of each unit make a shallow ridge that is levelled to the desired height by the adjustable shoe (E). The insecticide, delivered by the revolving, fluted feed cylinder, passes down the face of the adjustable shoe and spreads out in an even band on the shallow ridge. The disk hillers (N) cover the insecticide with soil and the higher ridge so formed is levelled by the concave rollers. The amount of soil ultimately covering the insecticide is regulated by adjusting the pitch of the disk hillers. If the insecticide is to be applied at or below ground level, the front hillers and supporting framework are, of course, removed from the machine. With various sizes of pulleys, the machine will apply 30 to 250 lb. of granulated material per acre but operates most efficiently when applying about 50 lb. per acre.

DISCUSSION

The machine has been used successfully in Prince Edward Island for small-plot and field-scale tests. For an acre of crop, the ridges may be made and levelled and the insecticide applied in less than 30 minutes; this is about six times as fast as when the operations are performed separately. Not only does the machine place the insecticide evenly at various depths in the soil, but the concave rollers leave marks or ridges showing exactly where the insecticide is in the row, thus facilitating the placing of the seed or plants in any desired position in relation to that of the insecticide. Also, a two-row rutabaga seeder may be connected directly to the machine (Figure 2, A) so that the tasks of ridging, applying the insecticide, and sowing the seed may be accomplished in one operation.

The machine was designed as a two-row unit not only to speed up the operation but also to give better balance. A one-row unit that was first constructed and tested experimentally swayed from side to side and required an operator, walking behind, to steady it.

In small-plot and field-scale tests of the machine in 1957, totalling 12 acres of experimental area, applications of granular heptachlor at 4 to 5 lb., or aldrin at 6 lb., of toxicant per acre gave 84 to 97 per cent control of root maggots attacking rutabagas. From 70 to 100 per cent of the plants in untreated plots or sections of the fields were infested. The machine may be used to apply insecticides to the soil for control of other soil insects attacking row crops, such as the carrot rust fly in carrots, white grubs in strawberries and the seed corn maggot in corn, beans, and peas; and also to apply boron to control brown heart (water core) in rutabagas.

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